PHYLOGEOGRAPHY OF MOOSE (*Alces alces*): GENETIC SIGNATURES OF POPULATION HISTORY

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By

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ABSTRACT

Through analysis of mitochondrial DNA (mtDNA) sequences, I examined phylogeographic relationships among moose (*Alces alces*) from Europe, Asia, and North America and inferred historic population trends explaining present-day structure of genetic variance. Diversity of nucleotide composition in cytochrome *b* was low worldwide, with no variation detected among North American moose. The North American lineage was more closely related to European than to Asian lineages, indicating a recent colonization of North America and refuting the theory of eastern and western races of moose. An analysis of the control region provided greater resolution, which revealed similar yet more detailed patterns, including detectable variation within North America subspecies. Patterns of genetic variation among regional populations identified central Asia as the source of extant lineages of moose. Moreover, a recent coalescence was indicated, with the most recent common ancestor dating to the last ice age. Two historic expansions of moose populations were detected: an initial expansion in Eurasia coincident with an interstade of the last ice age, and a second expansion in eastern Asia and North America following the end of the last ice age. Data indicate a low effective population size in Eurasia during the peak of the last ice age followed by population and range expansion, likely facilitated by climate change. Haplotypes within North America formed a star phylogeny, indicative of recent expansion. Nucleotide and haplotype diversity were greatest in central North America and least in peripheral populations (Alaska, Colorado, and eastern North America). My data indicate a pattern of colonization consistent with a large central population providing founders for peripheral

populations, perhaps resulting from leptokurtic dispersal. Low diversity in Alaska indicated a bottleneck subsequent to colonization and recent population expansion. Establishment of regional populations through small numbers of founders combined with selection pressure for smaller body size likely led to morphological differentiation among regional populations and likely was adequate for rapid development of subspecies. Nucleotide and haplotype diversity were low in southeastern Alaska, but were high in neighboring areas of British Columbia; there was little sharing of haplotypes occurred despite close proximity, indicating recent admixture of separate colonizing populations.

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ACKNOWLEDGMENTS

This dissertation includes 4 chapters, each of which is a complete manuscript that either has been accepted for publication in a peer-reviewed journal or is destined for submission. The citation for each manuscript is given as a footnote on the first page of each chapter. Those citations indicate my co-authors, each of whom contributed significantly to the manuscript(s) on which they appear, but I am senior author on all chapters. I generated and analyzed all of the original data presented in this dissertation, with 1 exception. In Chapter 2, additional data that supplemented my own were contributed by co-authors Irina Udina and Alexei Danilkin, Russian Academy of Sciences. Their contribution was 19 haplotypes of a total of 192. I wrote the dissertation, and the ideas contained herein are mine, although my co-authors and other committee members have influenced them.

Funding for portions of this research was obtained from Alaska Department of Fish and Game, Federal Aid in Wildlife Restoration, the Boone and Crockett Club, and the Institute of Arctic Biology. The DNA core sequencing facility, which proved an invaluable tool for data acquisition, was developed with a grant from the National Science Foundation.

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INTRODUCTION

Alces is a monotypic genus; the sole extant species, *A. alces* (L.), has a circumboreal distribution (Geist, 1998). Origination of that genus likely occurred in Eurasia during the early Pleistocene (>1.5 million years ago; Thouveny and Bonifay, 1984), with a chronocline from *A. gallicus* to *A. latifrons*, and to *A. alces*, the prevailing view of speciation (Lister, 1987, 1993). Lister (1993) reported the earliest fossil remains in Eurasia attributable to *A. alces*, dated to approximately 100,000 years ago; furthermore, he placed the transition from *A. latifrons* to *A. alces* in Eurasia in the upper Pleistocene, perhaps 200,000 to 100,000 years ago. Guthrie (1995) implied a much later transition because of the paucity of fossil evidence for *A. alces* from the Pleistocene and the presence of its putative ancestor, *A. latifrons*, in Beringia as late as 35,000 years ago.

The date and demographic characteristics of the worldwide expansion of *A. alces* remain open to conjecture. Mikko and Andersson (1995) estimated time of divergence for moose of Sweden and North America at 350,000 to 165,000 years ago, based on variation in sequences of the mitochondrial control region. Furthermore, those authors documented low variability within a locus of the major histocompatibility complex (MHC) on both continents as well as similar amino-acid motifs among MHC alleles. Mikko and Andersson (1995) concluded that moose must have passed through a population bottleneck prior to the divergence of lineages Europe and North America. Ellegren et al. (1996) also reported low variability in MHC in moose from Sweden, but used variation within highly polymorphic minisatellite loci as an index of the age of a presumptive bottleneck. Those authors concluded that the levels of minisatellite variation

they observed could have been generated within a homogeneous population in as little as 10,000 to 50,000 years. Conversely, Hundertmark et al. (1992) documented moderate levels of allozyme diversity in moose from Alaska, which was not indicative of a severe bottleneck.

Factors affecting the expansion of moose across the northern hemisphere as well as the divergence of moose lineages likely were governed, to a large degree, by climate change in the late Pleistocene (Lister, 1993, Guthrie, 1995). The Wisconsinan (North American) and Weichselian (European) glaciations were the last extensive ice ages of the Pleistocene and were contemporaneous with the emergence of *A. alces*. Data from North America indicate the Wisconsinan glaciation lasted from 120,000 to 8,000 years ago, with 2 periods of maximum ice coverage occurring from 75,000 to 65,000 and from 23,000 to 18,000 years ago (Fulton et al. 1986, Dyke and Prest, 1987). Northern Europe had a similar history, and Fennoscandia was completely covered with ice at glacial maxima (Lundqvist, 1986). Between the two periods of maximum extent of ice, an interstade occurred from approximately 60,000 to 30,000 years ago, which was characterized by a variable but generally warmer climate and retreating ice sheets (Fulton et al. 1986, Arkhipov et al., 1986). During the most recent glacial maximum, sea level in the North Pacific Ocean was ≤100 m lower than today and the Bering land bridge was exposed (Elias et al., 1996), thereby creating Beringia, the land mass connecting Asia and North America. Glacial coverage was much more extensive in North America than in Eurasia and represented an effective barrier between Beringia and the remainder of the

continent. The land bridge flooded approximately 11,000 years ago as glaciers melted (Elias et al., 1996), effectively separating the 2 continents.

Alces alces likely became established in North America during the final retreat of the Wisconsinan ice sheets (Geist, 1987, Bowyer et al., 1991, Cronin, 1992, Guthrie, 1995). Cronin (1992) documented a lack of variation in mtDNA restriction fragments in North American moose and concluded that the population must be relatively young and that moose entered North America from Beringia in the late Wisconsinan. Bowyer et al. (1991) and Guthrie (1995) supported a post-Wisconsinan entry based on the paucity of modern moose in the fossil record in North America prior to approximately 9,000 years ago (Guthrie, 1990). Geist (1987, 1998) also favored a recent entry of moose to the New World, and suggested that North American moose originated in Beringia from an ancestral population that also gave rise to the morphologically similar population in the Russian Far East (Magadan Oblast and vicinity). Whether North American moose arose in Beringia or migrated from Asia, a recent and common ancestry of moose in Alaska and far-eastern Asia has been presumed, with some authors placing those populations within the same subspecies (Peterson, 1952, Kistchinski, 1974).

The early view of geographic variation in modern moose consisted of a western race (*A. a. alces*) inhabiting Europe and western Asia to the Yenisei River, and an eastern race (*A. a. americana*) inhabiting eastern Asia and North America (Flerov, 1952). Although the eastern race had been subdivided into as many as 7 subspecies (Peterson, 1952, Chernyavski and Domnich, 1989), Geist (1998) argued for recognition of only the original 2 subspecies because they represented a fundamental phylogenetic division

within the species. Existence of distinct eastern and western races was supported by differences in morphology (Flerov, 1952, Peterson, 1952, Geist, 1987) and numbers of chromosomes. A karyotype of 2n = 68 typified European moose (Gustavson and Sundt, 1968), whereas 2n = 70 was characteristic of North American moose (Hsu and Benirscke, 1973). Moreover, the presence of a 75-bp repeat (indel) in the mitochondrial control region distinguished moose from Sweden and North America (Mikko and Andersson, 1995). Recently, the karyotype and form of mitochondrial indel associated with North American moose were documented in eastern Asia (Boeskorov, 1996, 1997, Udina et al., In press), further bolstering the notion of 2 races with ranges meeting in central Asia. Based primarily on karyotypes, Groves and Grubb (1987) defined eastern and western races of moose as semispecies, whereas Boeskorov (1997) proposed separate species status.

To better understand the demographic history of moose in Asia, as well as the colonization of North America and subsequent diversification of regional populations, I studied nucleotide variation within DNA sequences of mitochondrial markers. I selected the mitochondrial genome for those analyses because of its nonrecombining mode of transmission and fast evolutionary rate (Avise et al., 1987). Moreover, the control region has been useful in constructing phylogenies of other species of deer (Douzery and Randi, 1997, Polzeihn and Strobeck, 1998). I studies the cytochrome-*b* gene (Chapter 1), and the left hypervariable domain of the control region (Chapters 2-4) to discern phylogeographic patterns in populations of moose. I tested hypotheses that: 1) mtDNA variation would support 2 primary lineages of moose; 2) timing of divergence and

expansion of extant moose lineages would date to the most recent glacial period; 3) genetic evidence of a late-Pleistocene bottleneck exists; 4) moose occurring on either side of the Bering Sea (Magadan Oblast and North America) would share a recent Beringian ancestry; 5) the structure of geographic variation within North American moose would indicate a recent colonization consistent with serial founder events; and 6) diversity within and among currently recognized subspecies in North America would support those taxonomic designations.

CHAPTER 1[1]

GENETIC RELATIONSHIPS DEDUCED FROM CYTOCHROME-*b* SEQUENCES AMONG MOOSE

Abstract: We studied variation in nucleotide sequences of the mitochondrial cytochrome *b* gene to assess the phylogeny of moose (*Alces alces*) in general, and the position of North American moose within that phylogeny in particular. We combined North American, Asian, and European haplotypes generated for this study with 3 Eurasian haplotypes obtained from GenBank. No nucleotide variation occurred within moose from North America, whereas 3 haplotypes were present in European moose and 4 haplotypes in Asian moose. Clade structure was consistent over 4 most-parsimonious trees, with Asian haplotypes composing 1 clade, and North American and European haplotypes composing a second, albeit poorly supported clade. Low diversity of nucleotides in cytochrome *b* indicated a recent ancestry among moose worldwide. Existence of a single North American haplotype is strong evidence of a single, recent entry into the New World via the Bering land bridge, rather than multiple entries through ≥1 corridors. Furthermore, no phylogenetic support existed for the theory of distinct lineages of European versus Asian-North American moose.

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INTRODUCTION

Moose (*Alces alces*) arose in Eurasia in the late Pleistocene (Lister 1993), but paleontological (Guthrie 1990) and genetic (Cronin 1992) evidence indicate a recent colonization of North America. Such a recent colonization would result in characteristic genetic signatures in mitochondrial DNA (mtDNA), a haploid genome that is transmitted maternally, and is informative for constructing population histories of closely related taxa (Avise et al. 1987). Cronin (1992) analyzed restriction fragment length polymorphisms (RFLP) of mtDNA in North American cervids and reported that moose were unique among that group because they exhibited no detectable variation. There was no comparison to Eurasian moose in that study, however, to determine the degree of difference between moose inhabiting different continents. Therefore, the significance of those findings is difficult to assess.

Flerov (1952) described morphological variation in moose that distinguished western (Europe-western Asia) from eastern (Asia-North America) forms. Groves and Grubb (1987) used those differences as justification for labeling eastern and western moose as "semispecies." Eastern and western races coincide generally with differences in karyotype (Boeskorov 1996, 1997), and a length mutation in mtDNA (Mikko and Andersson 1995, Udina et al. in press). Geist (1998) supported the hypothesis of eastern and western races except to specify that the southern Asian subspecies, *A. a. cameloides*, belonged to a primitive regional fauna and could be considered as a valid subspecies. If those differences are based on systematic divergence between races of moose, phylogenetic analysis should support that hypothesis.

Cytochrome *b* has been used successfully to examine intraspecific genetic relationships in other North American mammals (Talbot and Shields 1996, Demboski et al. 1998). Moreover, we chose analysis of mtDNA nucleotide sequences rather than RFLP analysis because the former technique has the potential for yielding phylogenies of higher resolution than the latter (Hillis and Moritz 1990). Accordingly, we analyzed nucleotide variation within a section of the mitochondrial cytochrome-*b* (cyt*b*) gene to examine geographic distribution of genetic variation within moose, and to construct a phylogeny for this cervid. Cyt*b* evolves at a moderate rate in mammals (Irwin et al. 1991) and, consequently, often is used in phylogenetic studies of conspecific and congeneric taxa. We tested the hypotheses that variation within North American moose would be less than variation in Eurasian moose, and that a phylogeny of moose would distinguish between eastern and western races.

STUDY AREAS

Tissue samples were solicited from moose hunters in Alaska as well as biologists from across North America, Europe, and Asia. Samples were grouped to comprise ≥1 population from the range of each North American subspecies, and those populations were combined into continent-level associations. North American subspecies and sampling locations were: *A. a. gigas* ($n = 34$) from the across its range in Alaska; *A. a. andersoni* from central North America (USA: Minnesota, North Dakota, Isle Royale in Michigan; Canada: western Ontario and Manitoba; $n = 8$); *A. a. shirasi* from Colorado, USA ($n = 2$); and *A. a. americana* from New Hampshire, USA and New Brunswick, Canada ($n = 7$). The Colorado population originated from 3 translocations of moose from

neighboring states: 12 animals (8 females) from the Uinta Mountains, Utah, USA, in 1978, 12 animals (11 females) from Grand Teton National Park, Wyoming, USA, in 1979, and 12 animals (10 females) from Jackson Hole, Wyoming in 1987 (Duvall and Schoonveld 1988). Asian subspecies and sources were: *A. a. burturlini* (n = 10), which consisted of animals from the Ola Peninsula near Magadan, and the Omolon and Chelomya Rivers, Russia; *A. a. cameloides*, represented by 1 animal housed at a zoo in Harbin, China, and a sequence from GenBank (accession no. AY035872); and *A. a. pfizenmayeri*, consisting of a sequence obtained from GenBank (accession no. AY035873). The European subspecies, *A. a. alces*, consisted of samples collected in Finland (n = 6) and Sweden (n = 6), as well as a sequence of a moose from Norway obtained from GenBank (accession no. AJ000026; Randi et al. 2000).

MATERIALS AND METHODS

Tissue samples consisted of skeletal muscle, liver, kidney, skin, blood, or hair. Tissues were stored temporarily at -20°C or preserved in 100% ethanol as soon as possible after collection and were archived at -80°C. All tissue types except blood were subjected to salt extraction for isolation of genomic DNA. MtDNA was isolated from nuclear DNA and RNA from 1 moose by means of a $CsCl_2$ density-gradient centrifugation. That sample was used to verify the mitochondrial origin of amplified sequences.

We targeted the 5' region of the cyt*b* gene for analysis. We amplified the sequence with primers MVZ05 and MVZ04 (Smith and Patton 1993). Double-stranded templates were amplified in a reaction mix containing 100 mM Tris-HCl, pH 8.3, 500

mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 10 μM each primer, and 0.5 units DNA polymerase. Cycling conditions were a 2-min soak at 94°C followed by 30 cycles of 94°C (15 sec) denaturation, 50°C (15 sec) annealing, and 72°C (45 sec) extension, followed by one extension period of 10 min at 72°C. PCR products were visualized on a 6% agarose gel with ethidium bromide staining. Cleaned PCR products were cycle sequenced (both directions) with fluorescing ddNTPs. Nucleotide composition of the final products was determined on an automated sequencer (ABI 373, PE Applied Biosystems, Foster City, CA) with standard protocols supplied by the manufacturer. Sequences were aligned with the CLUSTAL V algorithm (Higgins et al. 1992) and were edited by visual examination of electropherograms with SEQUENCE NAVIGATOR software (ABI). All haplotypes identified in this study were submitted to Genbank.

We compared nucleotide sequences of individuals and identified sites at which they differed; those data served as the basis for describing individual and population-level variation. Populations also were characterized by haplotype diversity (*H*), which is the probability that 2 randomly selected individuals would have different haplotypes (Nei and Kumar 2000). Nucleotide diversity (π; Nei and Li 1979), which is the probability that 2 homologous nucleotides would differ in 2 randomly chosen individuals, and number of pairwise differences, which is the number of nucleotide substitutions observed between 2 haplotypes, also were determined. Those statistics were estimated with ARLEQUIN software (Schneider et al. 2000). Genetic distances between pairs of populations were computed by applying the Kimura 2-parameter model of sequence evolution (Kimura 1980). Differentiation among all populations was assessed via Φ_{ST},

which incorporates differences in nucleotide and haplotype diversity within and among populations. We analyzed relationships among haplotypes with a maximum parsimony (branch-and-bound search) cladogram, and a neighbor-joining tree (Saitou and Nei 1987) employing 2-parameter distance estimates. Those analyses were performed with the program MEGA version 2.0 (Kumar et al. In press). Trees were rooted by sequences from caribou and reindeer (*Rangifer tarandus*) and fallow deer (*Dama dama*) obtained from GenBank. Confidence in the structure of the phylogenies was assessed through 1,000 bootstrap replicates (Felsenstein 1985).

RESULTS

We detected 8 variable sites within the 403 nucleotides of the 5' end of cyt*b*, defining 8 haplotypes (Table 1.1). Six of the variable sites were transitions and 2 were transversions; the transversions were restricted to European haplotypes. The overall transition:transversion ratio was 7:1. Six variable sites, including 1 transversion, were synonymous substitutions at the third position of codons, resulting in no substitutions of in amino acids in the gene product. In haplotype Europe3, however, 1 transversion occurred at the third position of the thirty-third codon and resulted in the substitution of the amino acid phenylalanine with leucine. The remaining substitution was a synonymous, first-position transition in Europe1.

We documented an extreme degree of differentiation among continents (Φ_{ST} = 0.89), with no haplotype occurring on >1 continent (Table 1.1). We identified 4 Asian, 3 European and 1 North American haplotypes. Pairwise differences among haplotypes ranged from 1 to 7 substitutions, and associated estimates of genetic distances ranged

from 0.2 to 1.8% (Table 1.2). Estimates of mean (± SD) haplotype diversity for Europe ($H = 0.60 \pm 0.13$) and Asia ($H = 0.56 \pm 0.11$) were similar, as were estimates of nucleotide diversity for haplotypes occurring within those continents (Table 1.3). The least genetic distance between continents was the comparison between Europe and North America, and the greatest was between Europe and Asia. Europe exhibited the greatest within-continent diversity (Table 1.3).

Haplotype diversity differed among subspecies (Table 1). Although extensive sampling among the 4 North American subspecies did not yield nucleotide diversity, the Eurasian subspecies for which we had >2 samples exhibited great diversity of haplotype composition. *A. a. burturlini* exhibited 4 haplotypes among 10 individuals, and *A. a. alces* exhibited 3 haplotypes among 13 individuals (Table 1.1). The 2 individuals from *A. a. cameloides* and the single individual from *A. a. pfizenmayeri* exhibited haplotypes that were present in *A. a. burturlini.*

Six most-parsimonious trees were resolved, each with 81 steps (consistency index = 0.91, retention index = 0.90). The consensus tree constructed from all most-parsimonious trees (Fig. 1.1a) exhibited 2 clades, each supported at 100%, consisting of a strictly Asian clade and a European-North American clade. The neighbor-joining tree exhibited an identical structure (Fig. 1.1b), although bootstrap support for the clades in both trees was weak.

DISCUSSION

Amount of nucleotide variation we observed in moose was low relative to other cervids. Within the same region of cyt*b*, Kuwayama and Ozawa (2000) reported 32

variable sites among 5 subspecies of North American elk and red deer (*Cervus elaphus*), and 13 variable sites within 6 subspecies of sika deer (*C. nippon*) restricted to the islands of Japan. The maximum number of substitutions between a North American and an Asian haplotype in moose was 4 (all transitions). Comparatively, the minimum difference between haplotypes of North American elk (*C. e. canadensis* = *C.e. nelsoni*) and Asian red deer (*C. e. kansuensis*) was 5 substitutions, 3 of which were transversions (Kuwayama and Ozawa 2000). The magnitude of that difference indicated that North American elk and Asian red deer have been separated longer than equivalent populations of moose. The fossil record supports that conclusion (Guthrie 1966, 1990).

Low levels of variability we measured in cyt*b* within continents, combined with the small genetic distances between continents indicates a recent common ancestry for moose worldwide. Moreover, lack of shared haplotypes between continents indicated independent expansions of those populations from small numbers of founders. Had those continental assemblages arisen from the same expansion, we would have expected some sharing of haplotypes. Similarly, had the number of founding lineages in a continent been large, we would have expected more haplotypic diversity within continents. Patterns of variation we observed in moose from Asia and Europe were consistent, in each instance, with founding by a single lineage followed by the divergence of daughter lineages by 1 or 2 mutations.

Absence of variation in cyt*b* in North America is strong evidence for a single colonization event characterized by a small effective size. The relatively large haplotype diversity observed in Asian moose likely would have resulted in >1 haplotype in North

America if >1 colonization event occurred or if the colonization wave was comprised of many moose. Moreover, in North America, moose showed no close relationship to moose from the Russian Far East, which was unexpected if those populations were colonized by the same population expansion. The North American haplotype was basal in the neighbor-joining tree, indicating that this haplotype was the least distant to the outgroup taxa and, therefore, likely was more closely related to the common mitochondrial ancestor of moose than were other taxa in the tree. That outcome indicates that the North American lineage likely arose separately from the eastern Asian lineages we sampled, and that lineages of moose likely exist in other parts of Asia that are ancestral to both North American and eastern Asian lineages.

Our cyt*b* tree indicated a closer relationship between North American and European haplotypes than between either of those and Asian haplotypes. Thus, no support existed in those trees for a fundamental division of moose into European and Asian-North American clades. Moreover, the sharing of 1 haplotype between *A. a. cameloides* and *A. a. burtulini* indicated recent divergence and provided no evidence of an extreme temporal separation of *A. a. cameloides* from other Asian subspecies.

An unusual feature of our data was the occurrence of transversions and an amino acid substitution in the European haplotypes. A preponderance of those relatively rare classes of mutation indicates a greater age for the lineages carrying them. Our rooted phylogenetic trees, however, indicated that the European clade was derived from more basal moose lineages. Therefore, we must assume that the European lineages carry derived states and the North American and Asian lineages carry ancestral states.

We failed to reject the hypothesis that Eurasian moose exhibited more diversity than moose from North America, and we find that the spatial distribution of diversity within cyt*b* supports the idea of establishment of continental or regional populations of moose via expansion from small numbers of founders. Moreover, our data indicate a single entry of moose into North America from Asia, and do not support a fundamental division of moose into European and Asian-North American clades.

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Table 1.1. Nucleotide variation within the first 403 nucleotides of the 5' end of the mitochondrial cytochrome *b* gene in moose. Only variable nucleotide positions are listed, and dots represent identity with the first sequence. All haplotypes were documented in moose from this study with the exception of Europe3, which was reported by Randi et al. (2000) from Norway. Nucleotide positions were numbered according to the bovine mitochondrial genome (Anderson et al. 1982), with the first nucleotide of cytochrome *b* numbered 14514. Distributions of haplotypes by sampling location and by subspecies classification also are indicated.

	Continent			Subspecies								Nucleotide position							
Haplotype	North America	Asia	Europe	*A. a. americana*	*A. a. andersoni*	*A. a. shirasi*	*A. a. gigas*	*A. a. burturlini*	*A. a. cameloides*	*A. a. pfizenmayeri*	*A. a. alces*	14523	14559	14577	14595	14613	14652	14739	14872
North America	55			26	20	2	7					C	T	T	T	T	T	C	C
Asia1		8						7		1		T	C	•	•	•	•	T	•
Asia2		3						1	2			T	C	•	•	•	•	•	•
Asia3		1						1				T	C	•	•	•	C	T	•

Table 1.1 (continued)

Asia4	1		1		T	C	•	•	•	C	•	•
Europe1		4		4	•	•	A	•	•	•	•	T
Europe2		8		8	•	•	A	C	•	•	•	•
Europe3		1		1	•	•	A	C	G	•	•	•

Table 1.2. Genetic distances between pairs of haplotypes for a 403-nucleotide segment of the moose cytochrome *b* gene. Values above the diagonal are total numbers of substitutions, and those below the diagonal are estimates of substitutions per site using Kimura's (1980) 2-parameter model.

	North America	Asia1	Asia2	Asia3	Asia4	Europe1	Europe2	Europe3
North America		3	2	4	3	2	2	3
Asia1	0.008		1	1	2	5	5	6
Asia2	0.005	0.002		2	1	4	4	5
Asia3	0.010	0.002	0.005		1	6	6	7
Asia4	0.008	0.005	0.002	0.002		5	5	6
Europe1	0.005	0.013	0.010	0.015	0.013		2	3
Europe2	0.005	0.013	0.010	0.015	0.016	0.005		1
Europe3	0.007	0.015	0.013	0.018	0.015	0.007	0.002	

Table 1.3. Uncorrected distances between continental assemblages of moose haplotypes estimated with cytochrome *b* (below diagonal). Values in bold along the diagonal are estimates of nucleotide diversity within those assemblages. Kimura's (1980) 2-parameter model of sequence evolution was used in all calculations.

	North America	Asia	Europe
North America	**0.000**		
Asia	0.007	**0.002**	
Europe	0.005	0.012	**0.003**

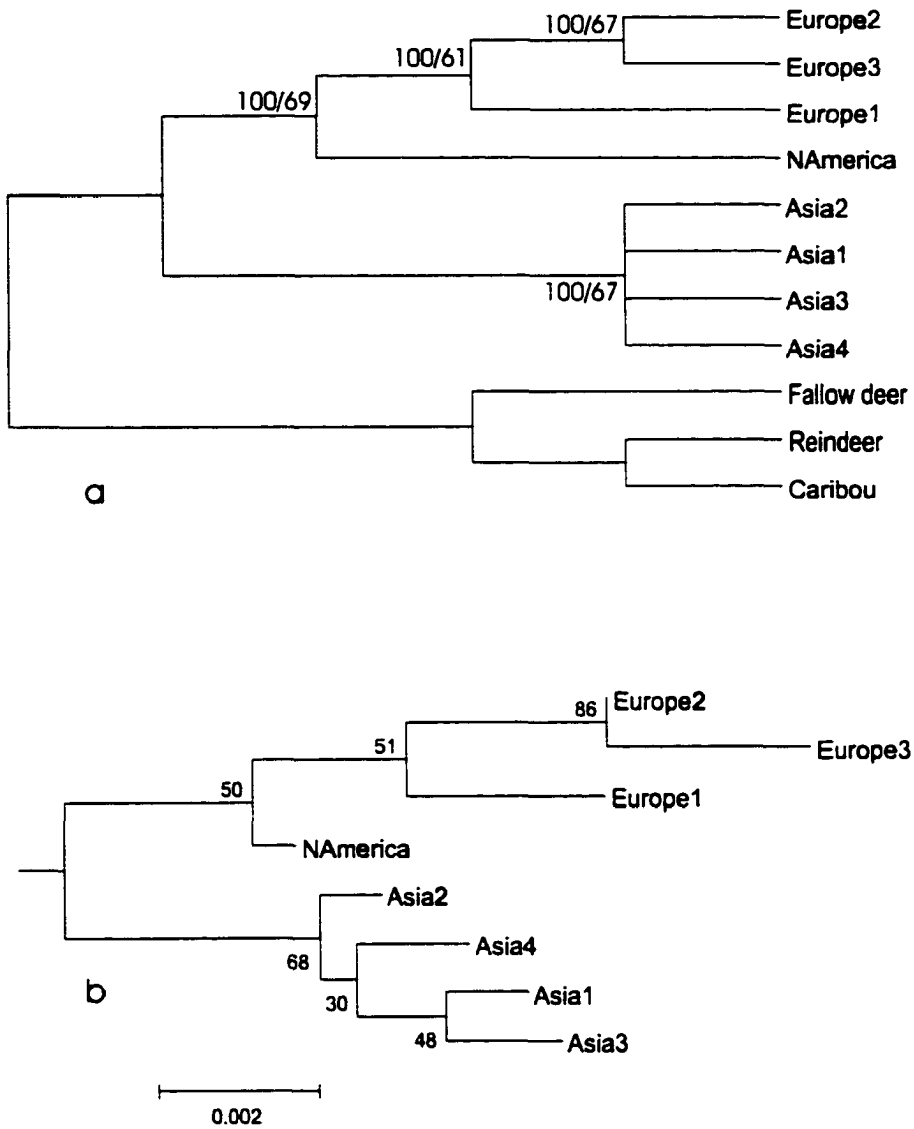

Fig. 1.1. Phylogenetic trees representing relationships among cytochrome-b haplotypes for moose worldwide. (a) Maximum parsimony cladogram rooted by fallow deer (*Dama dama*), reindeer (*Rangifer t. tarandus*), and caribou (*R. t.granti*) present percentage of most parsimonious trees conforming to that topology and bootstrap support for that node. (b) Neighbor-joining phylogram, with bootstrap support indicated at nodes.

CHAPTER 2[2]

MITOCHONDRIAL PHYLOGEOGRPAHY OF MOOSE (*Alces alces*): LATE PLEISTOCENE DIVERGENCE AND POPULATION EXPANSION

Abstract: We examined phylogeographic relationships of moose (*Alces alces*) worldwide to test the proposed existence of two geographic races, and to infer the timing and extent of demographic processes underpinning the expansion of this species across the Northern Hemisphere in the late Pleistocene. Sequence variation within the left hypervariable domain of the control region occurred at low or moderate levels worldwide and was structured geographically. Partitioning of genetic variance among regions indicated isolation by distance as the primary agent for differentiation of moose populations, but does not support the existence of distinct eastern and western races. Levels of genetic variation and structure of phylogenetic trees identify Asia as the origin of all extant mitochondrial lineages. A recent coalescence is indicated, with the most recent common ancestor dating to the last ice age. Moose have undergone two episodes of population expansion, likely corresponding to the final interstade of the most recent ice age and the onset of the current interglacial. Timing of expansion for the population in the Yakutia-Manchuria region of eastern Asia indicates that it is one of the oldest populations of moose, and may represent the source of founders of extant populations in North America, which were colonized within the last 15,000 years. Our data suggest an

[2] Hundertmark, K. J., G. F. Shields, I. G. Udina, R. T. Bowyer, A. A. Danilkin, and C. C. Schwartz. 2002. Mitochondrial phylogeography of moose (*Alces alces*): late Pleistocene divergence and population expansion. *Molecular Phylogenetics and Evolution* (In press).

extended period of low population size or a severe bottleneck prior to the divergence and expansion of extant lineages, and a recent, less-severe bottleneck among European lineages. Climate change during the last ice age, acting through contraction and expansion of moose habitat and the flooding of the Bering land bridge, undoubtedly was a key factor influencing the divergence and expansion of moose populations.

INTRODUCTION

Alces is a monotypic genus; the sole extant species, *A. alces* (L.), has a circumboreal distribution (Geist, 1998). Origination of this genus likely occurred in Eurasia during the early Pleistocene (>1.5 million years ago; Thouveny and Bonifay, 1984), with a chronocline from *A. gallicus* to *A. latifrons*, and to *A. alces*, the prevailing view of speciation (Lister, 1987, 1993). Lister (1993) reported the earliest fossil remains in Eurasia attributable to *A. alces*, dated to approximately 100,000 years ago; furthermore, he placed the transition from *A. latifrons* to *A. alces* in Eurasia in the upper Pleistocene, perhaps 200,000 to 100,000 years ago. Guthrie (1995) implied a much later transition due to the paucity of fossil evidence for *A. alces* from the Pleistocene and the presence of its putative ancestor, *A. latifrons*, in Beringia as late as 35,000 years ago.

The date and demographic characteristics of the worldwide expansion of *A. alces* remain open to conjecture. Mikko and Andersson (1995) estimated time of divergence for moose of Sweden and North America at 350,000 to 165,000 years ago, based on variation in sequences of the mitochondrial control region. Furthermore, they documented low variability within a locus of the major histocompatibility complex (MHC) on both continents as well as similar amino acid motifs among MHC alleles.

Those authors concluded that moose must have passed through a population bottleneck prior to the divergence of European and North American forms. Ellegren et al. (1996) also reported low variability in MHC in moose from Sweden, but used variation within highly polymorphic minisatellite loci as an index of the age of a presumptive bottleneck. They concluded that the levels of minisatellite variation they observed could have been generated within a homogeneous population in as little as 10,000 to 50,000 years. Conversely, Hundertmark et al. (1992) documented moderate levels of allozyme diversity in moose from Alaska, which was not indicative of a severe bottleneck.

Factors affecting the expansion of moose across the northern hemisphere as well as the divergence of moose lineages were governed, to a large degree, by climate change in the late Pleistocene (Lister, 1993, Guthrie, 1995). The Wisconsinan (North America) and Weichselian (Europe) glaciations were the last extensive ice ages of the Pleistocene and were contemporaneous with the emergence of *A. alces*. Data from North America indicate the Wisconsinan glaciation lasted from 120,000 to 8,000 years ago, with two periods of maximum ice coverage occurring from 75,000 to 65,000 and from 23,000 to 18,000 years ago (Fulton et al. 1986, Dyke and Prest, 1987). Northern Europe had a similar history, and Fennoscandia was completely covered with ice at glacial maxima (Lundqvist, 1986). Between the two periods of maximum extent of ice, an interstade occurred from approximately 60,000 to 30,000 years ago, which was characterized by a variable but generally warmer climate and retreating ice sheets (Fulton et al. 1986, Arkhipov et al., 1986). During the most recent glacial maximum, sea level in the North Pacific Ocean was ≤100 m lower than today and the Bering land bridge was exposed

(Elias et al., 1996), creating Beringia, the land mass connecting Asia and North America. Glacial coverage was much more extensive in North America than in Eurasia and created an effective barrier between Beringia and the remainder of the continent. The land bridge flooded approximately 11,000 years ago as glaciers melted (Elias et al., 1996), effectively separating the two continents.

Alces alces likely became established in North America during the final retreat of the Wisconsinan ice sheets (Geist, 1987, Bowyer et al., 1991, Cronin, 1992, Guthrie, 1995). Cronin (1992) documented a lack of variation in mtDNA restriction fragments in North American moose and concluded that the population must be relatively young and that moose entered North America from Beringia in the late Wisconsinan. Bowyer et al. (1991) and Guthrie (1995) supported a post-Wisconsinan entry based on the paucity of modern moose in the fossil record in North America prior to 9,000 years ago (Guthrie, 1990). Geist (1987, 1998) also favored a recent entry of moose to the New World, and suggested that North American moose originated in Beringia from an ancestral population that also gave rise to the morphologically similar population in the Russian Far East (Magadan Oblast and vicinity). Whether North American moose arose in Beringia or migrated from Asia, a recent and common ancestry of moose in Alaska and far-eastern Asia has been presumed, with some authors placing those populations within the same subspecies (Peterson, 1952, Kistchinski, 1974).

The early view of geographic variation in modern moose consisted of a western race (*A. a. alces*) inhabiting Europe and western Asia to the Yenisei River, and an eastern race (*A. a. americana*) inhabiting eastern Asia and North America (Flerov, 1952).

Although the eastern race had been subdivided into as many as seven subspecies (Peterson, 1952, Chernyavski and Domnich, 1989), Geist (1998) argued for recognition of only the original two subspecies because they represented a fundamental phylogenetic division within the species. Existence of distinct eastern and western races was supported by differences in morphology (Flerov, 1952, Peterson, 1952, Geist, 1987) and numbers of chromosomes. A karyotype of 2n = 68 typified European moose (Gustavson and Sundt, 1968), whereas 2n = 70 was characteristic of North American moose (Hsu and Benirscke, 1973). Moreover, the presence of a 75-bp repeat in the mitochondrial control region distinguished moose from Sweden and North America (Mikko and Andersson, 1995). Recently, the karyotype and form of mitochondrial indel associated with North American moose were documented in eastern Asia (Boeskorov, 1996, 1997, Udina et al., In press), further bolstering the notion of two races with ranges meeting in central Asia. Based primarily on karyotypes, Groves and Grubb (1987) defined eastern and western races of moose as semispecies, whereas Boeskorov (1997) proposed separate species status.

To better understand the history of moose in the late Pleistocene, we studied variation within the left hypervariable domain of the mitochondrial control region to discern phylogeographic patterns in populations worldwide. We chose the mitochondrial genome for those analyses because of its nonrecombining mode of transmission and its fast evolutionary rate (Avise et al., 1987); moreover, the control region has been useful in constructing phylogenies of other deer species (Douzery and Randi, 1997, Polzeihn and Strobeck, 1998). We tested the hypotheses that: a) mtDNA variation would support two primary lineages of moose; b) timing of divergence and expansion of extant moose

lineages would date to the most recent glacial period; c) evidence of a late-Pleistocene bottleneck exists; and d) moose occurring on either side of the Bering Sea (Magadan Oblast and North America) would share a recent Beringian ancestry.

MATERIALS AND METHODS

Tissue Collection and Population Identification

Tissue samples were solicited from moose hunters and biologists from across North America, Europe, and Asia (Fig. 2.1). North American populations were: A) northern, interior and southcentral Alaska (n = 62), B) southeastern Alaska and northwestern British Columbia (n = 23), C) Colorado (n = 19), D) central North America (n = 24), and E) eastern North America (n = 13). Asian populations were: F) Magadan Oblast (n = 18), G) Yakutia (n = 7), H) Eastern Asia (n = 7), consisting of Amur Oblast, Khabarovsk Kray, and northwestern China. European populations were: I) Finland (n = 10), J) Sweden (n = 6), and K) eastern Europe (n = 3), consisting of southern Urals (K_1) and Smolensk (K_2), Russia.

Tissue samples consisted of skeletal muscle, liver, kidney, skin, blood, or hair. Genomic DNA was extracted using standard protocols (Maniatis et al., 1989). Mitochondrial DNA was isolated from nuclear DNA and RNA from one moose by means of a $CsCl_2$ density-gradient centrifugation. That sample was used to verify the mitochondrial origin of amplified sequences.

DNA Amplification

Amplification and sequencing of samples were conducted in either of two laboratories (USA or Russia), with each laboratory having its own protocol. Protocol A:

primers LGL283 (L15693, 5'--TACACTGGTCTTGTAAAC--3') and ISM015 (H00068, 5'--ATGGCCCTGTAGAAAGAAC--3'); PCR conditions of a 2-min soak at 94°C followed by 30 cycles of 94°C (15 sec), 50°C (15 sec), and 72°C (45 sec) with a final extension period of 10 min at 72°C; cycle sequencing was conducted in both directions with fluorescing ddNTPs and final products were analyzed on an automated sequencer (ABI 373, PE Applied Biosystems). Protocol B: primers LmPro (L15766, 5'--GCCATCAACTCCCAAAGCT--3') and TDKD (H00074, 5'--CCTGAAGTAGGAACCAGATG--3'); PCR conditions of a 1-min soak at 95°C followed by 30 cycles of 95°C (10 sec), 62°C (20 sec), and 74°C (20 sec) with a final extension period of 5 min at 74°C; sequencing was conducted in both directions with the fmole DNA Cycle Sequencing System (Promega) and sequences were transcribed manually from autoradiograms. Primer binding sites were identified by strand and position, where H and L represent heavy and light strand, respectively, and the numerals represent the position of the 3' end of the primer relative to the appropriate strand of the bovine mitochondrial sequence (Anderson et al. 1982). Both protocols amplified the left hypervariable domain of the control region by having a light-strand primer within the flanking $tRNA^{Pro}$ gene and a heavy-strand primer within the central conserved domain of the control region. Protocol A amplified a slightly longer fragment than protocol B. All sequences were aligned with the CLUSTAL V algorithm (Higgins et al., 1992). Indels were aligned, whenever possible, to correspond with similar indels in other species of deer (Douzery and Randi, 1997). After alignment, the longer sequences were truncated to match the shorter sequences, and all sequences were truncated to remove the 3' end of

$tRNA^{Pro}$. Sequences of all haplotypes were submitted to GenBank and were accession numbers AF412224–AF412269, and AF414123.

Data Analysis

Phylogenetic relationships. A neighbor-joining (Saitou and Nei, 1987) tree of haplotypes was computed with Kimura's (1980) two-parameter model of sequence evolution. A maximum parsimony cladogram was constructed with a heuristic search, with gaps treated as a fifth state, and any gap larger than one base was treated as a single entity. PAUP* version 4.0b4 (Swofford, 1999) was used to construct the neighbor-joining and parsimony trees, and confidence in those topologies was assessed by 1,000 bootstrap replicates (Felsenstein, 1985). A maximum likelihood phylogeny was computed with program PUZZLE version 4.0 (Strimmer and von Haesler, 1996), and the reliability of the topology was assessed by 10,000 quartet-puzzling steps. The Hasegawa-Kishino-Yano (Hasegawa et al., 1985) model of sequence evolution was chosen for the maximum likelihood approach. Trees were rooted with homologous sequences from elk (*Cervus elaphus*; Polziehn and Strobeck, 1998) and Chinese water deer (*Hydropotes inermis*; Douzery and Randi, 1997) that were obtained from GenBank, and caribou (*Rangifer tarandus*; this study). Evolutionary relationships among haplotypes also were inferred from a minimum spanning tree constructed with ARLEQUIN version 2.0 (Schneider et al., 2000). That method used a parsimony approach to connect each sequence to its closest neighbor, based on pairwise differences, and differed from traditional methods of tree construction by allowing extant haplotypes to occupy internal nodes.

Inter- and intrapopulation variation. Estimates of variability were computed with ARLEQUIN and MEGA2 (Kumar et al., 2001). We expressed variation within populations as haplotype diversity (H), nucleotide diversity (π), number of segregating sites (S), and average number of pairwise differences (d_X) among haplotypes assuming a Kimura (1980) two-parameter model of sequence evolution. Genetic differentiation between populations was expressed as mean number of pairwise differences per site (d_{XY}), and as pairwise Φ_{ST}, which took into account variation in haplotype frequencies among populations as well as genetic distance based on nucleotide variation. Distribution of genetic variance within a hierarchical structure of population organization was expressed as Φ-statistics in a nested analysis of molecular variance (AMOVA; Weir and Cockerham, 1984, Excoffier et al., 1992). We conducted AMOVA analysis with three groups representing current continental populations (Europe, Asia, and North America) and with two groups representing the putative eastern (Asia and North America) and western (Europe) lineages of moose. Statistical confidence in variance estimates was determined by comparing the observed Φ-statistics against a distribution of estimates generated from 10,000 permutations of data (Excoffier et al., 1992). We used a Mantel test (Mantel, 1967) to determine if pairwise values of Φ_{ST} were related to geographic distances between populations; North America was considered a single population for that analysis with a geographic location in Alaska.

We estimated net pairwise divergence per base pair (d_A), which is proportional to time since divergence (T) of two populations. Divergence time was calculated as $T = d_A / 2\mu$, where μ is the rate of nucleotide substitution (Nei and Kumar, 2000). An

appropriate rate had not been calibrated for moose, so we used two different estimates for divergence (2μ), each of which was specific to the left hypervariable domain of the control region. One estimate was 62.8% per million years derived for domestic cattle (Brady et al., 1996), and the other was 78.5% per million years calculated for *Bison* (Burzynska et al., 1999).

Historic demography. We tested for the presence of historic population bottlenecks with Tajima's *D*-statistic (Tajima, 1989a,b). Historic demographic expansions were detected by examination of frequency distributions of pairwise differences of sequences (mismatch distributions) within continental or regional assemblages (Slatkin and Hudson, 1991, Rogers and Harpending, 1992). Concordance of our data with the distribution underlying the sudden-expansion model of Rogers (1995) was assessed by means of a least-squares approach (Schneider and Excoffier, 1999) implemented by ARLEQUIN. For distributions that did not differ significantly ($P > 0.05$) from the expectations of the sudden-expansion model, we estimated τ (an estimate of the mode of the mismatch distribution), which is an index of time since expansion expressed in units of mutational time (Slatkin and Hudson, 1991). Confidence intervals for τ were estimated from 10,000 bootstrap replicates. We transformed values of τ to estimates of time since expansion with the equation $\tau = 2\mu t$, where μ is the mutation rate for the sequence, and t is the time since expansion. We used the same estimates of mutation rates described previously for divergence estimates.

RESULTS

Sequence Variation

The section of the control region we analyzed was 442 to 518 nucleotides in length, with the first nucleotide in the moose control region corresponding to nucleotide position 15792 in the bovine mitochondrial genome (Anderson et al., 1982). Variation in length was attributable to two length mutations: a single insertion in six Eurasian haplotypes at position 510, and the 75-bp indel. The fragment comprising the large indel was absent in all North American moose as well as four Asian moose from Yakutia and eastern Asia, yielding the shorter sequence. There were four variable sites within the large indel: three transitions and one transversion. The transversion occurred in all moose from eastern Europe and in three of 18 moose from Magadan Oblast. The first 365 nucleotides of the sequence (excluding the large indel) comprised the left hypervariable domain of the control region. Excluding indels, there were 47 polymorphic sites within the fragment we analyzed: 46 transitions and one transversion. That transversion was confined to one individual from eastern Europe. No site had more than two bases segregating.

We identified 16 haplotypes among 141 individual moose sampled from North America. Twenty-five different haplotypes were detected among 51 Eurasian moose: 19 in 32 Asian moose and six in 19 European moose. No haplotypes were shared among continents, and there was little sharing of haplotypes among populations within continents. Among all haplotypes, $\pi = 0.025$ and $d_X = 10.64$. The number of pairwise

differences between haplotypes ranged from 1-23 (1-25 if indels were included). Among all individuals, $H = 0.919$, $\pi = 0.018$, and $d_X = 8.05$.

Phylogenetic Relationships

The neighbor-joining tree exhibited a shallow branching structure with four clades that we grouped into three phylogroups (Fig. 2.2) corresponding roughly to continental associations. Asian haplotypes occurred in each clade, and one phylogroup was exclusively Asian (phylogroup 1). European (phylogroup 2) and North American (phylogroup 3) haplotypes were confined to separate phylogroups. The maximum likelihood and parsimony trees (not shown) formed nearly identical topologies except that the maximum likelihood tree joined the two clades comprising phylogroup 1 with 50% bootstrap support. Phylogroups 1 and 2 were characterized by the presence of the 75-bp insertion. Absence of that fragment was a reliable marker for membership in phylogroup 3, because haplotype composition of that phylogroup was identical in all trees even though the indel was used as an informative character in the parsimony analysis only. One European haplotype exhibited an unusually long branch (Fig. 2.2); that haplotype occurred in all moose from Sweden. In pairwise comparisons among all haplotypes, the Swedish haplotype was ranked as most divergent from 32 of the remaining 40 haplotypes.

The minimum-spanning tree (Fig. 2.3) was the most parsimonious arrangement of haplotypes assuming only one loss or gain of the large indel. That tree shows phylogroups 2 and 3 connected to a central phylogroup 1. Net sequence divergence between those groups ranged from 1.7 to 2.5%, whereas mean pairwise differences

within groups ranged from 0.69 to 1.20% (Fig. 2.3); phylogroup 3 exhibited the lowest level of within-group variation. Phylogroups 1 and 2 were equally divergent from phylogroup 3, suggesting some degree of homoplasy. Asian haplotypes within phylogroup 3 occurred in Yakutia and eastern Asia. Haplotypes sampled from Magadan Oblast, which was the closest Asian population geographically to North America, were associated with phylogroups 1 and 2, but not phylogroup 3. Phylogroup 3 exhibited a star-like structure indicative of recent or rapid expansion. Based on divergence rates of 62.8% and 78.5% per million years, we estimated divergence of phylogroups 1 and 3 at approximately 27,000 and 21,500 years ago, respectively. Similarly, our estimates for divergence of phylogroup 2 and phylogroup 1 were approximately 38,000 and 30,000 years ago.

Inter- and Intrapopulation Variation

Pairwise comparisons of Φ_{ST} indicated significant ($P < 0.05$) differentiation between populations except for the comparison between Yakutia and eastern Asia (Table 2.1). For further analyses, those populations were combined to form a population we termed Far East (Fig. 2.1). Measures of population divergence generally were greatest between North America and populations of Europe, and least between North America and populations of Asia (Table 2.1). Pairwise estimates of Φ_{ST} between populations were related significantly to geographic distance ($r = 0.37$, $P = 0.023$; Fig. 2.4). That relatively low correlation, however, was influenced to a large degree by the Sweden population, which showed a high degree of divergence from all populations regardless of geographic distance (Fig. 2.4). When that population was removed from the analysis, the

relationship between Φ_{ST} and geographic distance improved ($r = 0.67$, $P = 0.011$). The comparison between North America and Magadan Oblast was notable in that analysis (Fig. 2.4), with a large amount of differentiation for the moderate geographic separation.

Asian populations of moose exhibited the greatest nucleotide diversity, followed by European, and North American populations (Table 2.2). Moose from Asia also exhibited more segregating sites than those in North America or Europe, and had the greatest haplotype diversity. When Asian moose were segregated into two populations, Far East exhibited much more diversity than did Magadan Oblast. Moose from Europe were the next most diverse, but much of the heterogeneity therein related to the highly divergent haplotype from Sweden. The levels of nucleotide diversity in moose of eastern Europe and Finland were similar to that in Magadan Oblast and North America (Table 2.2).

Analysis of molecular variance demonstrated a high degree of structure at all levels of organization (Table 2.3). The index of differentiation among groups (Φ_{CT}) was greater for the three-group comparison (Asia vs. Europe vs. North America) than for the two-group comparison (Europe vs. Asia + North America), and was associated with a corresponding decrease in differentiation among populations within groups (Φ_{SC}; Table 2.3). If the two-race hypothesis were correct, we would have expected the opposite result.

Historic Demography

We detected no signature of historic bottlenecks; values of Tajima's *D* were nonsignificant ($P > 0.05$) for all populations (Table 2.2). The mismatch distribution for

moose worldwide exhibited two distinct waves, which we attributed to temporally distinct expansions in Eurasia and North America (Fig. 2.5). Mismatch distributions for North America, Eurasia, Magadan Oblast, and Far East did not differ significantly ($P > 0.05$) from expectations under the sudden-expansion model and therefore were suitable for analysis of demographic patterns. The mismatch distribution for Europe differed significantly from the sudden-expansion model ($P = 0.038$). North America and Magadan Oblast showed evidence of recent expansions, whereas the Far East expansion was older. Estimates of τ indicated that Eurasian moose underwent an expansion that was 4.2 times older than that undergone by moose in North America. Relative ages of expansion for populations in Far East and Magadan Oblast (as multiples of North America) were 3.1 and 0.1, respectively. The 95% confidence intervals of τ did not overlap in comparisons between Eurasia and North America, or between Far East and Magadan Oblast (Fig. 2.5) indicating significantly different times since expansion for those populations.

Our estimates of time of expansion for Eurasian moose, based on the two rate estimates, were approximately 59,000 and 47,000 years ago, whereas our estimates for North American moose were approximately 14,000 and 11,000 years ago (Table 2.4). Expansion of the Far East population occurred early in the expansion of Eurasian moose, but the moose population in Magadan Oblast was of very recent origin.

DISCUSSION

Despite their extensive distribution throughout the northern part of the Northern Hemisphere, moose exhibit a distinct lack of diversity within the control region compared

to other species of large, hoofed mammals. Nucleotide diversity for reindeer (*R. tarandus*), a species with a distribution similar to moose, was 3.42% (Gravlund et al., 1998), compared to our estimate for moose of 1.8%. Nucleotide diversity for the central African antelope, kob (*Kobus kob*), was 4.6%, with a maximum intrapopulation diversity of 5.72% (Birungi and Arctander, 2000), greatly exceeding our estimates for moose. Moreover, nucleotide diversity across the range of moose was less than or equal to that of four species of African bovids sampled from only portions of their ranges (Arctander et al., 1996b), and the greatest level of intrapopulation diversity in moose (2.1% in Far East) was less than that measured in 16 of 27 populations in three species of bovids distributed across southern Africa (Arctander et al., 1996a). Those comparisons indicate a demographic history of moose consisting of low effective size and recent expansion.

Our data indicate an extensive expansion of moose populations at the end of the late Pleistocene in Eurasia from which all extant populations were derived. The three phylogroups we documented diverged within a short period; divergence of phylogroups 1 and 2 likely occurred first, followed by the splitting of phylogroups 1 and 3. Expansion dates estimated from mismatch distributions corresponded generally with dates of divergence of the three phylogroups in the gene tree and support the hypothesis of Guthrie (1995) that modern moose flourished only recently. The paraphyly of Asian haplotypes with respect to European and North American haplotypes suggests Asia as the region of origin for all extant lineages of moose. Subsequently formed populations in Europe and North America have not been separated long enough from the founding Asian lineages, however, to achieve reciprocal monophyly.

The expansion of moose in Magadan Oblast and North America most likely occurred after the last glacial maximum. Nonetheless, the lack of close relationship between those two populations is notable and unexpected. If the wave of moose that colonized North America left remnant populations in its path, a close relationship between Magadan Oblast and North America would be expected. Perhaps genetic drift caused the differences we demonstrated, but the estimate for expansion of the Magadan Oblast population indicates that population is recent, and is not descended directly from the wave that colonized North America. Thus, we conclude that the noted morphological similarity between moose from Magadan Oblast (*A. a. burturlini*) and Alaska (*A. a. gigas*) resulted from convergent evolution. Gravlund et al. (1998) documented a similar instance of polyphyletic origins of morphologically similar populations. In that instance, three subspecies of small-bodied, high-arctic reindeer (*R. tarandus*) were shown to have arisen from two different lineages. Furthermore, a common Beringian ancestry for North American and Siberian moose was not supported by paleontological data (Sher, 1987, Guthrie, 1990), which indicate that moose were not present in northeastern Asia or Beringia in great numbers much earlier than 9,000 years ago. Consequently, we reject the hypotheses of Geist (1987, 1998) and Cronin (1992) for place of origin for the moose population in North America.

Of the populations we sampled, Far East was the Eurasian population most closely related to North America as a whole, and is the best candidate as the source for the colonizers of North America. Our estimates of the timing of that colonization were similar to estimates of a colonization of North America by humans based on control

region variation (Bonatto and Salzano, 1997). Remarkably, studies place the origin of aboriginal human populations of North America in the same region as for moose (Neel et al., 1994, Bonatto and Salzano, 1997).

The presence of *A. latifrons* in eastern Beringia 35,000 years ago (Harington, 1978, Guthrie, 1990) indicated that species existed either parapatrically with *A. alces* in Asia during the late Pleistocene or disappeared at the time that *A. alces* flourished in Asia. In either instance, we note that the most recent confirmed dates for *A. latifrons* in North America coincide with our estimate for the date of expansion of Eurasian moose. Beringia probably was the last refuge for *A. latifrons*, but *A. alces* eventually replaced it.

Assuming that *A. alces* existed in Eurasia for the last 100,000 years, the limited diversity and recent divergence of lineages that we have documented must be interpreted as evidence of either an extended period at low effective size, or a bottleneck that constrained the genetic diversity of modern moose. Our estimate of the timing of that occurrence is more recent than the estimate of 350,000-165,000 years ago (Mikko and Andersson, 1995), and is consistent with the conclusion of Ellegren et al. (1996) that moose in Sweden could have generated the observed level of genetic diversity within 50,000 years. Our divergence estimates were more in line with the paleontological record, as the estimate of Mikko and Andersson (1995) markedly predates the earliest known evidence of *A. alces* (Lister 1993). Certainly, divergence of a single marker can predate the divergence of taxa that carry it. If that were true in this instance, either a multiregional origin of moose combined with substantial gene flow, or a large effective population size at the *A. latifrons –A. alces* transistion would be suggested. Given the

high degree of female philopatry exhibited by moose (Hundertmark, 1998), we believe that the multiregional explanation is unlikely. Furthermore, the paucity of sequence variation present in modern moose argues against a large historic N_e.

The primary reason for the difference in estimates of divergence between Mikko and Andersson (1995) and this study was the faster mutation rates we used in our calculations. We selected those rates because they applied only to the left hypervariable domain of the control region (the range of our data), and were derived from taxa closely related to moose. Moreover, the estimates we used were well within the bounds of short-term rates estimated for humans, which can be as high as 110% per million years (Lundstrom et al., 1992). Nonetheless, dates assigned to historic events based on rates of genetic divergence are subject to much variation, and should be interpreted with caution. Yet, application of a more conservative rate of 33% per million years (Ward et al., 1991) still placed our oldest estimate for coalescence of moose worldwide within the last 85,000 years.

Historic Effective Size

Our inability to detect a bottleneck was informative but may not indicate the true demographic history of moose. Tajima's *D* tests for an expected divergence of *S* and π caused by bottlenecks (Tajima 1989b), but is thought to have low statistical power (Hartl and Clark, 1997, Nei and Kumar, 2000). If a population went through a severe bottleneck such that virtually all variation was purged, Tajima's *D* likely would not detect that event. Moreover, a population that existed at low effective size for a long period

might not express such a signature. Thus, we have no conclusive evidence of an historic bottleneck, but cannot reliably discount its existence.

Some insight may be gained into historic demographics by estimating historic effective population size (N_e). According to Neigel and Avise (1986), the effective size of a population can be predicted with respect to the phylogenetic relationships among regional populations. When one population is paraphyletic with respect to another, there is a high probability that the clades diverged between N_e and $4N_e$ generations ago. That model required an estimate of the time of founding of the daughter populations, which we could not estimate for Europe. Nonetheless, that event must have occurred after the divergence of phylogroups 1 and 2, which was approximately 34,000 years ago. If we use that estimate, however, and a generation time of 7 years, we estimate N_e within a range of 1,214 to 4,856 female moose. For North America and Asia, we used the midpoint of our estimates of the expansion of North America (~13,000 years ago), and estimated a range for N_e of 464 to 1,856 females. Those numbers suggest both regions were founded by a small effective number of female moose.

In a qualitative assessment of demographics, we note that Magadan Oblast and North America have moderate to high H and low π, a signature of rapid demographic expansion from a small effective population size, and Far East has large H and large π, a characteristic of a stable population. Conversely, Europe was characterized by relatively low H and low π, a signature of a recent bottleneck (Avise, 2000). Excoffier and Schneider (1999) modeled the effects of bottlenecks on mismatch distributions, and the distribution we observed for Europe was similar to the results of one of their simulations.

In that example, Excoffier and Schneider (1999) imposed a recent bottleneck on a population that had previously undergone sudden expansion. They demonstrated that such a scenario would cause rejection of the sudden expansion model by the least-squares test even when an expansion had occurred. Consequently, European moose may have suffered a recent reduction in effective size after a late-Pleistocene expansion. Historical accounts of moose in Fennoscandia (Markgren, 1974) indicated a marked decline in moose numbers beginning in the 15th century; furthermore, intense exploitation of moose by humans in the 18th and 19th centuries led to near extirpation of that species in Norway, Sweden, and Finland. A slow recovery was not completed until the mid-20th century when commercial forest activities created abundant habitat for moose, and extirpation of predators allowed maximal population growth (Markgren, 1974). Therefore, such scenario is plausible.

Characteristics of Moose Lineages

The structure of the gene tree and the similar magnitude of difference of pairwise Φ_{ST} estimates between Europe-Asia and North America-Asia indicated that mitochondrial markers offered no support for two primary (eastern and western) lineages of moose; rather, those data are more consistent with an isolation-by-distance process of divergence. That conclusion also was supported by AMOVA results; the three-lineage scenario maximized variation among groups and minimized variation within groups.

The haplotype found in moose from Sweden was the only exception to the isolation-by-distance model. We hypothesize that the divergence of that haplotype was a result of drift associated with isolation. A migration corridor existed from western

Europe directly into southern Sweden because of lower sea levels in the late Pleistocene (Taberlet et al., 1998). That corridor and a second path through Finland were proposed by Markgren (1974) as routes by which moose colonized Sweden and Norway. Taberlet et al. (1998) documented a floral and faunal suture zone across central Norway and Sweden that supports the existence of those routes for other species as well. Thus, the haplotype from Sweden may represent a lineage of moose that colonized the region from the south, perhaps from a different glacial refugium than the one from which other lineages in Scandinavia arose.

The chromosomal differences noted between eastern and western moose must be of recent origin, occurring some time after the expansion of Eurasian moose but prior to the divergence of phylogroups 1 and 3. Nonetheless, those chromosomal differences may cause reproductive isolation in moose in central Siberia, which warrants further study. Presence or absence of the 75-bp repeat was not indicative of an eastern-western division because we documented both forms of the indel in moose from Asia. Furthermore, the amount of variation within the repeat, and the absence of the repeat in the most recently derived lineage lead us to conclude that the presence of the additional 75-bp fragment is the ancestral form in moose. Ultimately, that fragment originated as an insertion (Douzery and Randi, 1997), but the deletion of that fragment in moose from phylogroup 3 is the explanation most consistent with our data.

Paleoecological Factors Affecting Phylogeography of Moose

The glacial cycles of the Pleistocene had a dramatic effect on population structure of northern species (Hewitt, 1996, 2000, Bernatchez and Wilson, 1998, Taberlet et al.,

1998). Species inhabiting areas that recently were covered by Pleistocene ice sheets exhibit reduced levels of genetic diversity and shallow depth of clades compared with species further to the south (Bernatchez and Wilson, 1998). Mechanisms affecting the structure of those populations include latitudinal shifts of habitats, geographic barriers, number and location of refugia, and dispersal ability of the species (Hewitt, 1996, 2000). Moose represent an excellent illustration of these effects on a species that 1) is adapted to a habitat (boreal forest) that underwent large range shifts associated with climate change (Anderson and Brubaker, 1993); 2) has the potential for long-distance dispersal but normally exhibits a high degree of female philopatry (Hundertmark, 1998); and 3) utilized a route (Bering land bridge) to spread to North America that now is an effective barrier to gene flow.

The range of suitable habitat for moose in Eurasia in the late Pleistocene would have moved north and south with cooling and warming trends, even though Asia was relatively ice-free (Guthrie, 1995). Moreover, the east-west orientation of mountain ranges in Eurasia would have imposed limits to the southern spread of habitats, likely causing reductions in their size. The process of latitudinal shifts of range can reduce genetic heterogeneity in animal species restricted to affected habitats (Hewitt, 1996). The first cold phase of the most recent glacial period, which occurred from about 75,000 to 65,000 years ago according to data from North America (Fulton et al., 1986), provides the necessary conditions for reduction of population size in moose in Eurasia. That period saw extensive ice coverage in northern Europe (Forsström and Punkari, 1997), and the extent of ice coverage in Siberia was greater than during the most recent glacial

maximum (Arkhipov et al., 1986), which would have had a profound effect on the distribution of boreal forest. The relatively mild climate of the most recent interstade (approximately 60,000 to 30,000 years ago) most certainly was accompanied by expansion of Eurasian boreal forest and, subsequently, moose populations. Our estimates of expansion of Eurasian moose and the divergence of phylogroups 1 and 2 coincide with that period. The most recent cold period, 23,000 to 18,000 years ago (Dyke and Prest, 1987), saw another contraction of habitat and populations, and provided a mechanism for separate refugia in Asia and the subsequent divergence of phylogroups 1 and 3. Finally, climatic warming of the current interglacial caused the northward spread of boreal forest in Eurasia, allowing moose to enter Scandinavia by multiple pathways and to colonize North America via Beringia prior to the flooding of the land bridge 11,000 years ago (Elias et al., 1996). The narrow temporal window during which moose could have passed across the land bridge restricted opportunities for gene flow between Asian and North American populations, and likely was responsible for the star-like phylogeny of moose haplotypes from North America.

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Table 2.1. Indices of population differentiation for moose. Values above the diagonal are pairwise Φ_{ST}, and those below the diagonal are average net pairwise divergence (d_A) between populations. All values assume a Kimura (1980) two-parameter model of sequence evolution, and differ significantly ($P < 0.05$) from zero except where noted.

	North America	Finland	Eastern Europe	Sweden	East Asia	Yakutia	Magadan Oblast
North America		0.78	0.75	0.84	0.49	0.49	0.74
Finland	2.8		0.38	0.82	0.60	0.57	0.78
Eastern Europe	2.4	0.3		0.89	0.43	0.41	0.73
Sweden	4.2	1.3	1.6		0.70	0.72	0.84
East Asia	0.7	1.7	1.5	3.3		0.08[A]	0.34
Yakutia	0.7	1.3	1.4	3.0	0.2[A]		0.47
Magadan Oblast	2.3	2.8	2.6	4.1	0.6	1.2	

[A] $P > 0.05$

Table 2.2. Measures of intra-population variability for moose based on grouping at the continental level. Values in parentheses are variances of the estimates. Mutations within the indel were ignored.

Population/ Region	N	No. haplotypes	Haplotype diversity (*H*)	Nucleotide diversity (π)	Segregating sites (*S*)	Mean pairwise differences (d_X)	Tajima's *D*
North America	141	16	0.86 (0.02)	0.007 (0.004)	19	3.0 (1.6)	-0.29
Asia	32	19	0.94 (0.03)	0.019 (0.010)	39	9.6 (4.5)	0.08
Magadan Oblast	18	8	0.84 (0.07)	0.010 (0.006)	23	5.0 (2.6)	-0.84
Far East	14	12	0.98 (0.03)	0.021 (0.011)	33	11.0 (5.3)	0.39
Europe	19	6	0.74 (0.07)	0.010 (0.006)	18	5.3 (2.7)	0.31
Eastern Europe	3	2	0.67 (0.31)	0.008 (0.007)	6	4.0 (2.7)	0.00
Finland	10	3	0.38 (0.18)	0.004 (0.003)	8	2.2 (1.3)	-0.92
Sweden	6	1	0.00	0.00	0	0.0	0.00

Table 2.3. Analysis of molecular variance for moose based on grouping sequences by three continents (North America, Asia, and Europe) and by populations within continents. Probability values refer to variance components and Φ-statistics, and were generated by comparing observed values against the distribution of 1,000 permutations.

Groups	Source of variation	Percentage of variance	Φ	*P*
Europe vs. Asia vs. North America	Among groups	61.5	$\Phi_{CT} = 0.62$	<0.0001
	Among populations/ within groups	21.6	$\Phi_{SC} = 0.56$	<0.0001
	Within populations	16.9	$\Phi_{ST} = 0.83$	<0.0001
Europe vs. Asia + North America	Between groups	58.1	$\Phi_{CT} = 0.58$	0.009
	Among populations/ within groups	28.8	$\Phi_{SC} = 0.69$	<0.0001
	Within populations	13.1	$\Phi_{ST} = 0.87$	<0.0001

Table 2.4. Estimates of τ for the mismatch distributions of moose populations based on variation within the left hypervariable domain of the control region, and associated estimates of expansion times based on two different divergence rates. Confidence intervals were constructed using 10,000 bootstrapped data sets.

		0.628×10^{-6}		0.785×10^{-6}	
	τ	Expansion time (years ago)	95% CI	Expansion time (years ago)	95% CI
North America	3.87	13,942	5,044-29,181	11,154	4,035-23,345
Eurasia	16.40	59,083	30,622-84,301	47,266	24,498-67,441
Far East	12.30	44,312	26,299-60,524	35,450	21,039-48,419
Magadan Oblast	0.36	1,297	0-22,336	1,038	0-17,869

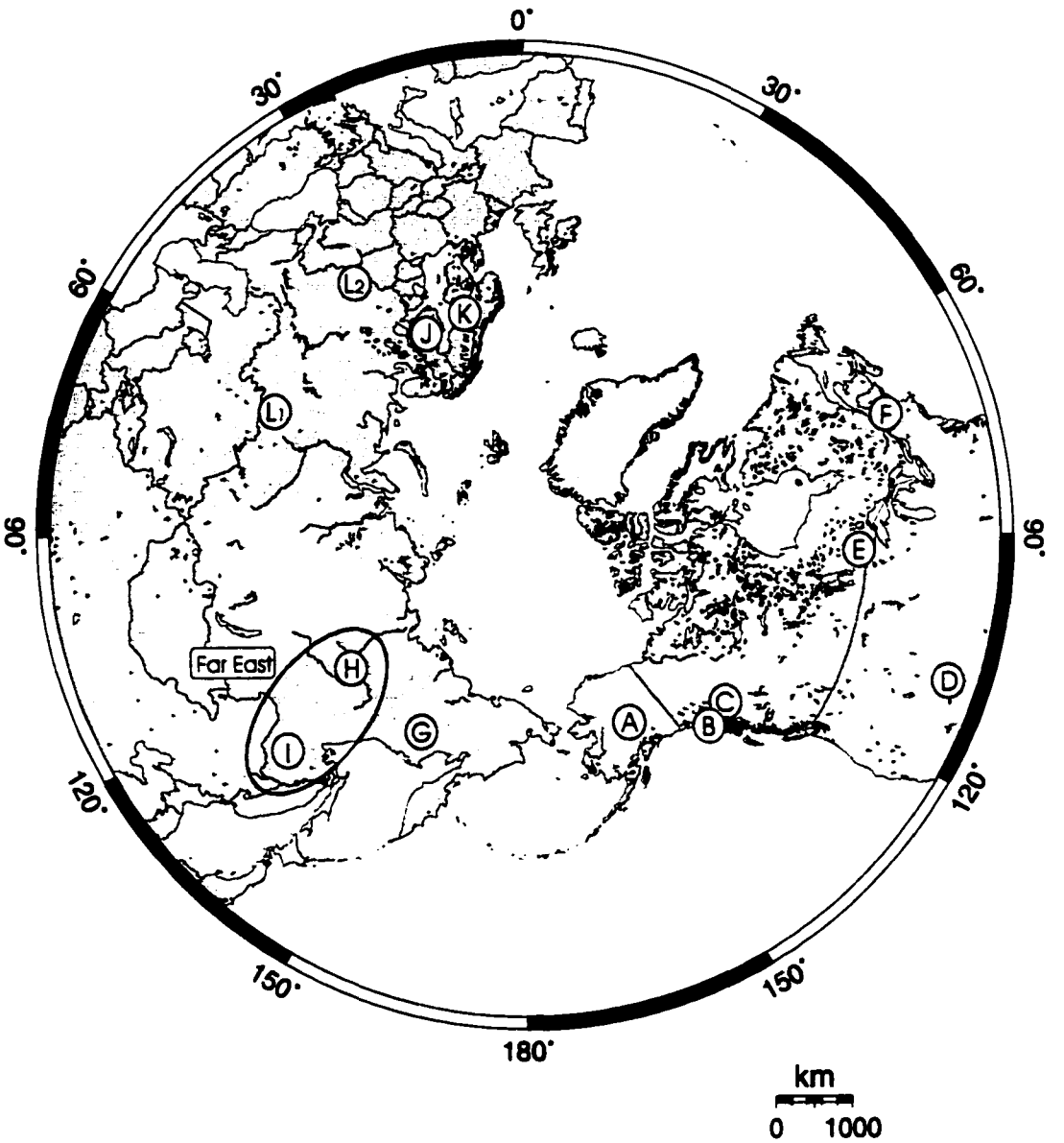

Fig. 2.1. Sampling sites for moose used in this study. Multiple sampling sites contained within a circle were combined to form a single population for particular analyses. The perimeter of the map is 35° north latitude.

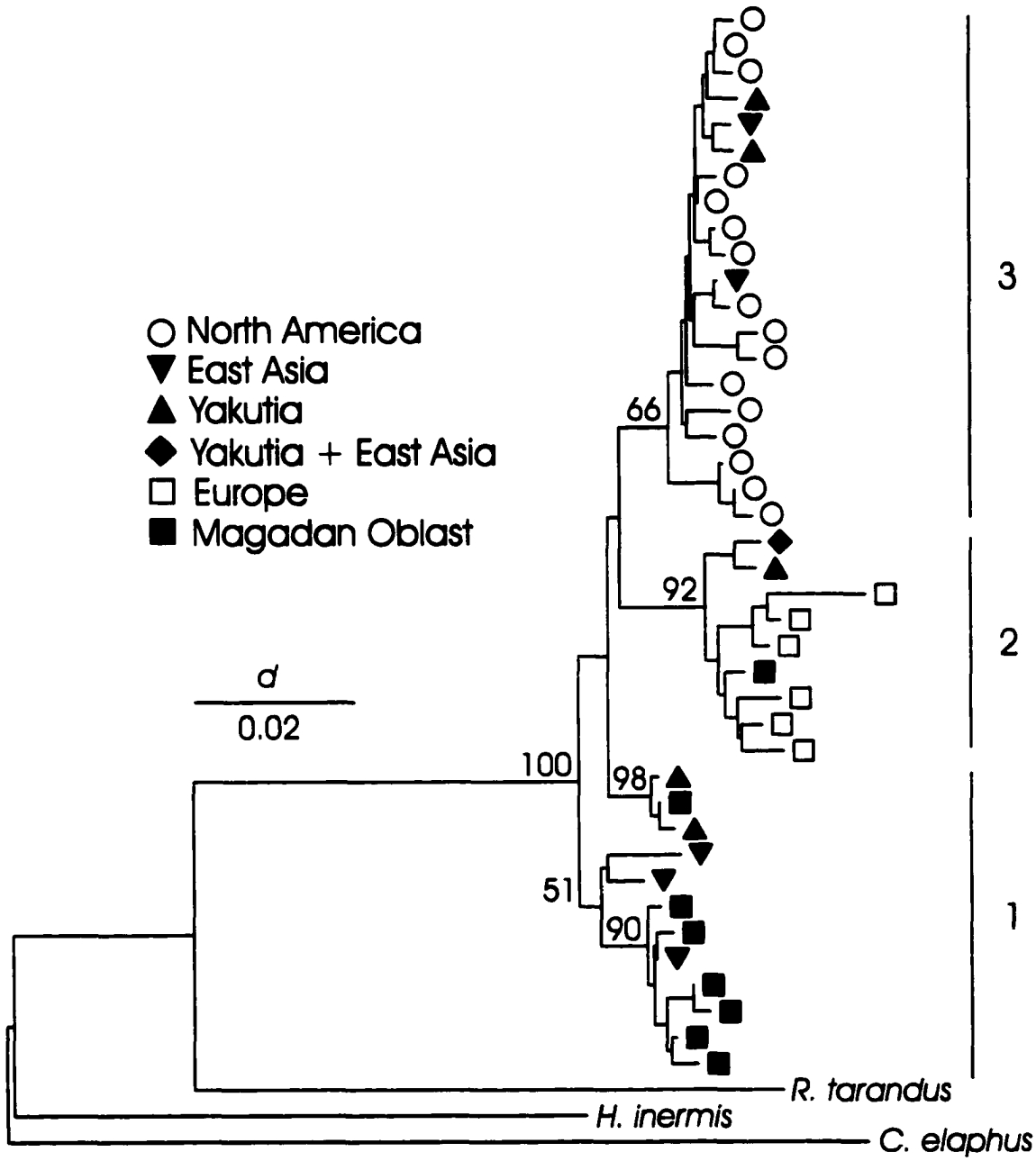

Fig. 2.2. The neighbor-joining tree representing phylogenetic relationships among control region haplotypes for moose worldwide. Bootstrap support for major branches are indicated at nodes. Phylogroup membership is indicated at the right.

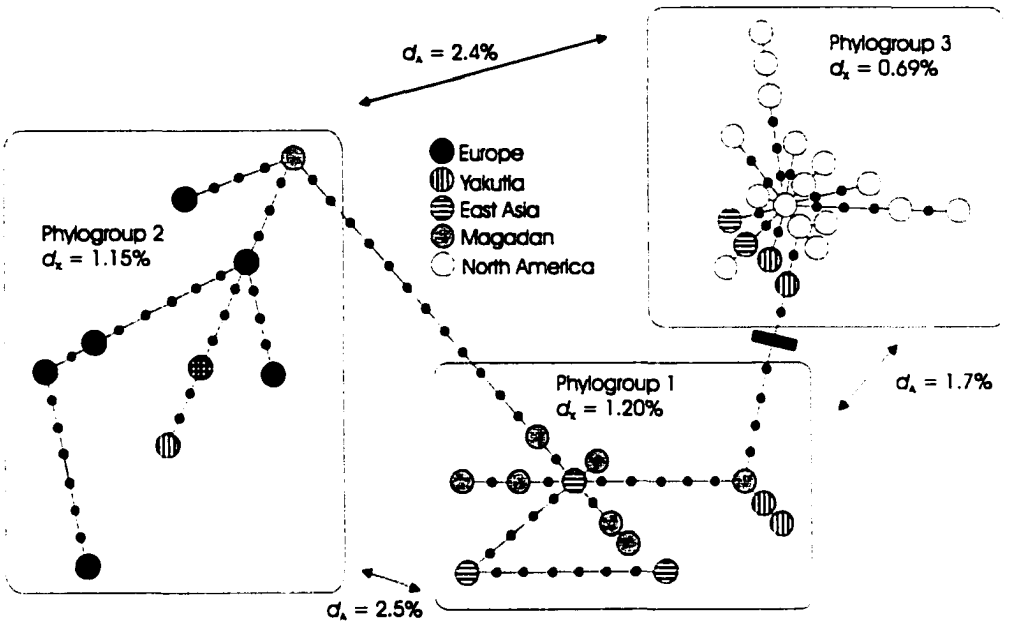

Fig. 2.3. A minimum spanning tree of worldwide moose haplotypes. Filled and open circles indicate haplotypes we analyzed whereas small black dots indicate presumptive intermediate haplotypes assuming a change of one substitution per step. The black bar indicates the branch on which the deletion of the 75-bp fragment occurred.

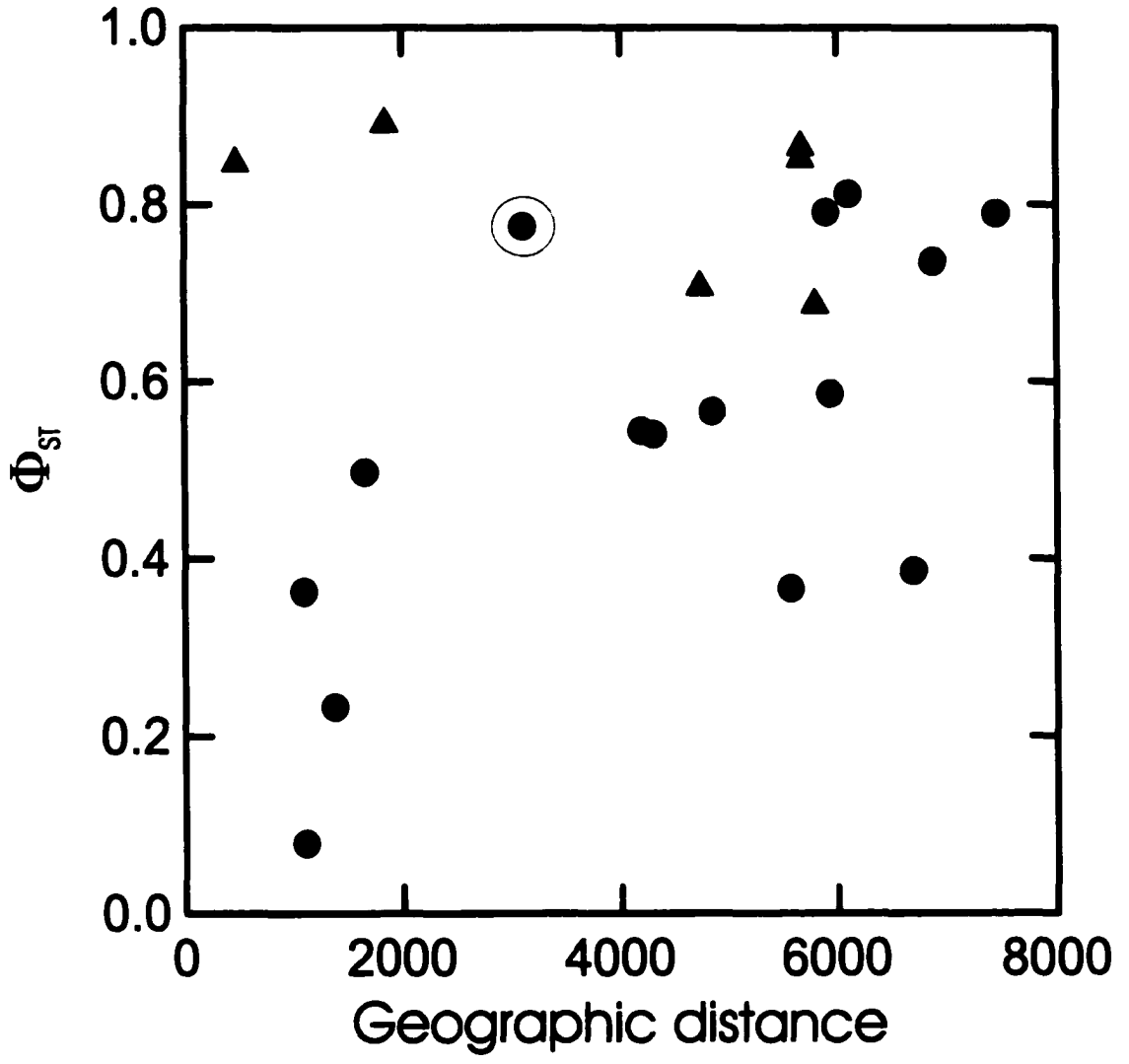

Fig. 2.4. Plot of pairwise comparisons of Φ_{ST} values versus geographic distance between moose populations. Comparisons involving the population from Sweden are indicated by triangles. The circled observation is the comparison between North America and its nearest Asian population, Magadan Oblast.

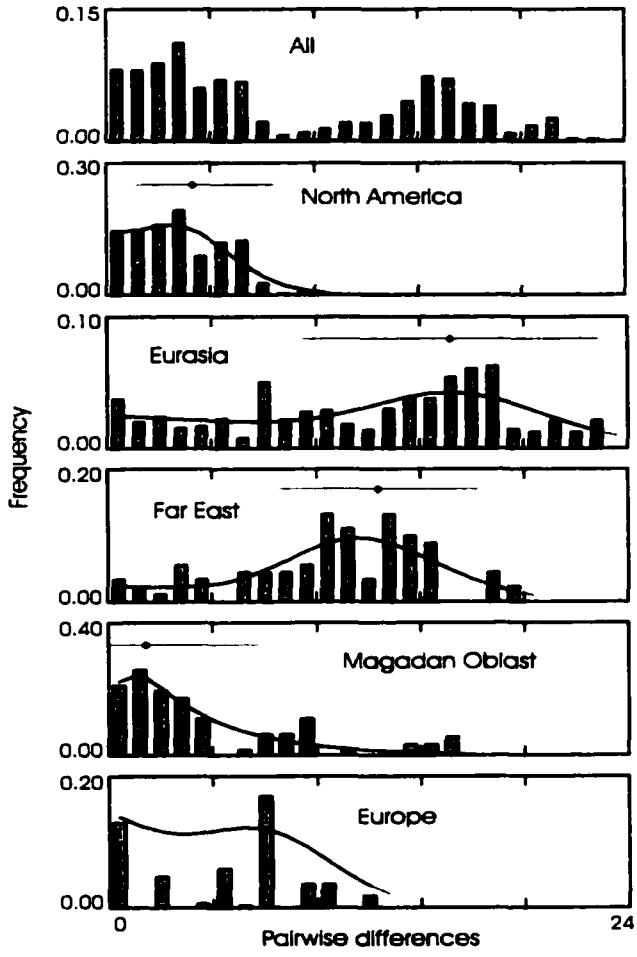

Fig. 2.5. Mismatch distributions for worldwide, continental or regional assemblages of moose, based on the 442-bp segment of the control region. The abscissa represents number of pairwise differences and the ordinate represents the proportion of observations. The vertical bars are the observed distribution of mismatches and the smoothed line represents the expected distribution under the sudden expansion model of Rogers (1995) as modified by Schneider and Excoffier (1999). For those continental or regional distributions that met the assumptions of the sudden expansion model, a horizontal bar is provided that shows the mean and 95% confidence interval for 5,000 bootstrapped estimates of τ, the mode of the distribution.

CHAPTER 3[3]

MITOCHONDRIAL PHYLOGEOGRAPHY OF MOOSE (*Alces alces*): RANGE EXPANSION AND SUBSPECIATION IN NORTH AMERICA

Abstract: We assessed nucleotide variation within the mitochondrial control region of North American moose (*Alces alces*) to test predictions of a model of range expansion by stepping-stone dispersal, and to determine if patterns of genetic variation support designation of 4 subspecies. Haplotypes formed a star phylogeny indicative of a recent expansion. Nucleotide and haplotype diversity were low continent-wide, but were greatest in the central part of the continent and least among peripheral populations. Despite low mitochondrial diversity, moose exhibited a high degree of differentiation regionally, which was not explained by isolation by distance. Our data indicate a pattern of colonization consistent with a large central population corresponding to the subspecies *A. a. andersoni* that supplied founders to peripheral populations other than *A. a. gigas*, perhaps through rare, long-distance dispersal events (leptokurtic dispersal) rather than mass dispersal via a stepping-stone model. Our colonization scenario does not account for the low haplotype diversity we observed in Alaskan moose (*A. a. gigas*), which may derive from a post-colonization bottleneck. Establishment of peripheral populations through leptokurtic dispersal, combined with selection, may have been sufficient for development of morphological differentiation among extant subspecies.

[3] Hundertmark, K. J., R. T. Bowyer, G. F. Shields, and C. C. Schwartz. Submitted. Mitochondrial phylogeography of moose (*Alces alces*): range expansion and subspeciation in North America. *Journal of Mammalogy*.

INTRODUCTION

The moose (*Alces alces*) is a recent immigrant to North America, having dispersed from Asia through Beringia about 14,000-11,000 years ago, shortly before the flooding of the Bering land bridge (Chapter 2). The process by which moose expanded their range across the continent, however, is still in doubt. Moreover, the process by which 4 North American subspecies were formed in conjunction with that expansion continues to be debated (Bubenik 1998a; Geist 1998).

Hundertmark et al. (1992) proposed a stepping-stone mode of colonization combined with serial founder effects, yielding a decreasing gradient of genetic diversity from the point of entry into North America (Alaska) to the eastern extent of the continent. Slatkin (1993) modeled scenarios of dispersal wherein a range expansion occurred very quickly under a stepping-stone model and time since expansion (τ) varied. Isolation by distance was not apparent when τ was small, but became more apparent as τ increased. Moose colonizing North America underwent a sudden population expansion in the early Holocene (Chapter 2) that may be sufficiently recent for such a mechanism to operate. Yet, under the scenario of recent expansion as modeled by Slatkin (1993), the genetic composition of pre- and post-expansion populations were similar, which is a different outcome than predicted by the hypothesis of serial founder events proposed by Hundertmark et al. (1992).

The 4 subspecies of moose recognized in North America (Peterson 1955, Hall 1981) inhabit Alaska and a portion of Yukon Territory (*A. a. gigas*), western Canada to the Great Lakes (*A. a. andersoni*), the Rocky Mountains from northern Colorado to

southern Alberta (*A. a.* shirasi), and eastern North America from the Great Lakes to the east coast (*A. a. americana*; Bowyer et al. in press). Some of those subspecies differ morphologically (Peterson 1955, Bowyer et al. 1991) and behaviorally (Molvar and Bowyer 1994, Bubenik 1998b). Based on anecdotal evidence, Peterson (1955) concluded that, as recently as the early 20th century, moose were still in the process of expanding their range after the retreat of ice sheets from the Wisconsinan glaciation. Thus, despite recent colonization, regional populations of moose may have been isolated, causing divergence through genetic drift and subsequent selection for adaptations for particular habitat characteristics. Geist (1998) argued, however, that morphological variation in North American moose is clinal and is not a basis for subspecific recognition.

We analyzed variation in nucleotide sequences from a hypervariable domain of the mitochondrial control region to detect population structure in North American moose. Specifically, we examined geographic variation in the mitochondrial genome to determine the circumstances under which moose expanded their range, and to test whether geographic variation provides support for the current designation of 4 subspecies. Although mtDNA variation alone is not sufficient to identify subspecies, that marker is informative for assessing population history and levels of gene flow among regions (Avise et al. 1987). Different colonization scenarios would produce different patterns of mtDNA variation. A stepping-stone scenario combined with serial founder events (Hundertmark et al. 1992) would produce a directional gradient of higher diversity in Alaska to lower diversity in populations toward the end of the colonization pathway. Wave-like colonization characterized by founding events with large effective sizes would

exhibit relatively homogeneous distribution of genetic variation, possibly combined with an isolation-by-distance pattern of diversity. We tested the hypothesis that genetic variation of populations would decrease with distance from Alaska, where moose colonized North America via the Bering land bridge (Chapter 2).

MATERIALS AND METHODS

Tissue samples were acquired from specimens contributed by successful moose hunters in all populations. We collected samples from Alaska (n = 74), northwestern British Columbia (n = 11), Colorado (n = 19), central North America (n = 24), and eastern North America (n = 13). Samples from moose in Alaska were obtained from throughout the range of the species and were divided geographically into "mainland" (*A. a. gigas*, n = 61) and "southeastern" (subspecies undetermined, n = 13) populations; the latter population was composed of moose from central and southern regions of the southeastern panhandle, but did not include an isolated population in Berners Bay that was established as a transplant of *A. a. gigas* (Burris and McKnight 1973). The population from British Columbia (*A. a. andersoni*) represented moose from areas immediately east of the coastal mountain range separating southeastern Alaska from Canada. Samples from Colorado (*A. a. shirasi*) were collected from an introduced population in Jackson County, which was founded by animals from up to 3 separate introductions from source populations in Utah and Wyoming (Duvall and Schoonveld 1988). The central North American population (*A. a. andersoni*) represented moose from northeastern and northcentral Minnesota, southwestern Ontario, Isle Royale, Michigan, northeastern North Dakota, and the Lake Winnipeg area of Manitoba. The eastern

population (*A. a. americana*) was composed of moose from New Hampshire and New Brunswick.

Tissue samples consisted of skeletal muscle, liver, kidney, or skin and were stored temporarily at -20° C or preserved in 100% ethanol as soon as possible after collection and then archived at -80° C. All tissue types were subjected to salt extraction for isolation of genomic DNA (Millar et al. 1988). We isolated mtDNA from nuclear DNA and RNA from 1 moose by means of a $CsCl_2$ density-gradient centrifugation (Sambrook et al. 1989). That sample was used to verify the mitochondrial origin of the amplified sequences.

The targeted sequence within the mtDNA molecule was the left hypervariable domain of the control region. That portion of the mitochondrial genome evolves at an extremely fast rate and is useful for constructing phylogenies in cervids (Douzery and Randi 1997), particularly among recently diverged taxa, and for intraspecific population studies (Avise et al. 1987). Primers LGL283 (5'-TACACTGGTCTTGTAAAC-3'), located within $tRNA^{Thr}$, and ISM015 (5'-ATGGCCCTGTAGAAAGAAC-3'), located within the central conserved domain of the control region, were used to amplify a mitochondrial sequence. Double-stranded templates were amplified via polymerase chain reaction (PCR) in a reaction mix containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphates (dNTPs), 10 μM for each primer, and 0.5 units DNA polymerase (AmpliTaq, Perkin Elmer). Cycling conditions were a 2-min soak at 94° C followed by 30 cycles of 94° C (15 s) denaturation, 50° C (15 s) annealing, and 72° C (45 s) extension, followed by 1 extension period of 10 min at 72° C.

PCR products were visualized on a 6% agarose gel with ethidium bromide staining. Primers, dNTPs, and polymerase were separated from successful PCR amplifications via precipitation in polyethylene glycol. Cleaned PCR products were then cycle sequenced (both directions) with fluorescing dideoxynucleotide triphosphates. Nucleotide composition of the final products was determined on an automated sequencer (ABI 373, PE Applied Biosystems, Foster City, CA) with standard protocols supplied by the manufacturer. We aligned sequences with the CLUSTAL V algorithm (Higgins et al. 1992) and edited electropherograms with SEQUENCE NAVIGATOR software (PE Applied Biosystems). The control region was identified within the amplified fragment by comparison with published sequences from other cervids (Douzery and Randi 1997). Sequences generated for this study were deposited in GenBank (accession nos. AF412224-AF412250).

We assessed relationships among haplotypes with a neighbor-joining phylogeny (Saitou and Nei 1987) computed with MEGA2 (Kumar et al. 2001). We also generated a minimum-spanning tree of haplotypes, which was useful for examining short-term evolutionary pathways among closely related haplotypes (Excoffier and Smouse 1994). That analysis applied a distance-parsimony approach to generate a tree with haplotypes occupying both internal nodes and branch tips. To generate that tree we used the software PAUP 4.0b8 (Swofford 1999) to infer sequence characteristics of haplotypes at internal nodes in a maximum- parsimony tree, and those inferred sequences were included with haplotypes we described to construct a distance matrix (Kimura 1980) using MEGA2. Those distances were used to generate the minimum-spanning tree with

ARLEQUIN 2.0 (Schneider et al. 2000). Estimates of variability of haplotype sequences within and among populations also were computed with ARLEQUIN. Sequence variation among individuals and within populations was expressed as haplotype diversity (*H*: the probability that 2 randomly chosen individuals will have different haplotypes), mean number of nucleotide differences in pairwise comparisons (d_X), and nucleotide diversity (π: the probability that 2 randomly chosen individuals will differ at a nucleotide site--Nei and Kumar 2000). The degree of structuring among populations was measured as Φ_{ST}, an analog of Wright's F_{ST} for use with DNA sequences, as estimated from a hierarchical analysis of molecular variance (AMOVA; Weir and Cockerham 1984; Excoffier et al. 1992) performed by ARLEQUIN. We conducted a Mantel test (Mantel 1967) to determine if significant correlation existed between genetic distances and geographic (great circle) distances among populations. Coancestry coefficients (Reynolds et al. 1983) were used as genetic distances between populations. We used ARLEQUIN to generate 1,000 sets of bootstrapped data, from which we determined the probability of observing a correlation coefficient less than or equal to that computed from original data.

We searched online databases for homologous sequences from moose with associated location information to compare with sequences generated for this study. Aside from a single haplotype from a moose collected in Banff National Park, Alberta, Canada (Polzeihn and Strobeck 1998; Genbank accession no. AF016951), we located none. Mikko and Andersson (1995), however, listed polymorphic sites for the control region of 19 Canadian and 30 Swedish moose. Although no location information was assigned to individual haplotypes, their samples from Canada were collected in 5 national

parks in the Rocky Mountains, 1 park in central Saskatchewan, and 1 park in Newfoundland. With the exception of the sample from the Newfoundland park (*A. a. americana*), all samples came from the range of *A. a. andersoni*, including its contact zone with *A. a. shirasi*. We compared our sequences with the table of polymorphic sites (Table 2 in Mikko and Andersson 1995), and by assuming that all other sites were identical to our consensus sequence, we obtained an alignment. We did not include those sequences in most analyses because they lacked specific locality data; rather, we treated them as an independent sample to determine if our sampling efforts were successful in describing existing variation.

To examine temporal and geographic extent of the colonization, we obtained records of fossil materials from North America that had been assigned radiometric dates (A. Dyke, in litt.). Histograms of occurrence of dated fossils were created for 4 contiguous regions of North America: >130° W longitude, 130°-110° W longitude north of 52° N latitude combined with 110°-80° W longitude, 130°-110° W longitude south of 52° N latitude, and <80° W longitude. Those regions approximated the ranges of *A. a. gigas*, *A. a. andersoni*, *A. a. shirasi*, and *A. a. americana*, respectively.

RESULTS

Sequence characteristics

The fragment of the control region we analyzed was 470 nucleotides in length, and was characterized by 20 variable sites (Table 3.1). All substitutions were transitions, and no deletions or insertions were detected. We identified 16 haplotypes among 142 North American moose. Although nucleotide diversity was low among individuals (π =

0.0069, $d_X = 3.23$) and haplotypes ($\pi = 0.010$ and $d_X = 4.87$), haplotype diversity was high continent-wide ($H = 0.86$).

Phylogenetic relationships

The rooted neighbor-joining tree contained 2 major clades--1 clade contained 3 haplotypes that were confined to southeastern Alaska and northwestern British Columbia (haplotypes C, D and G), whereas the other clade contained all other haplotypes (Fig. 3.1). Within the latter clade, haplotypes from Alaska, central North America, and eastern North America clustered together (haplotypes A, B, L, M, J, O and P–Fig. 3.1). The remaining haplotypes from central North North America, British Columbia and Colorado presented an unresolved branching structure.

The minimum-spanning tree (Fig. 3.2) indicated a star-like structure with haplotypes occurring on short branches radiating from a central haplotype. The longest branch was 6 haplotypes in length as measured from the central node (haplotype A). The root of the tree, as determined by inclusion of an outgroup (not shown), was haplotype J, which we observed only in central North America (Table 3.1).

Population variation and structure

Mitochondrial diversity varied among the North American populations. Populations in central North America and British Columbia exhibited the greatest diversity, whereas mainland Alaska, southeastern Alaska, and eastern North America showed little variation. Moose in the Colorado sample were monomorphic for a haplotype that occurred nowhere else (Table 3.2).

Matrices of geographic and genetic distances were not correlated significantly (r = 0.22, P = 0.29). Nonetheless, haplotypes were segregated strongly on a geographic basis. Thirteen of 16 haplotypes we identified were restricted to 1 population, and no haplotype occurred in >2 populations (Table 3.2). Overall, Φ_{ST} from AMOVA was 0.73 ($P <$ 0.0001). The mainland population from Alaska exhibited moderate levels of diversity, but those were inflated by inclusion of animals from the contact zone with southeastern Alaska. When that small zone of contact was excluded, the mainland portion of Alaska consisted of only 2 haplotypes that differed by 1 substitution. One of those haplotypes (A) also occurred in 1 individual in central North America, and that haplotype occupied the central position of the minimum-spanning tree (Fig. 3.2). In southeastern Alaska, we documented a unique motif consisting of 2 haplotypes (C and D) differing by a single substitution. A mixture of motifs, including mainland Alaska, southeastern Alaska, and central North America, characterized moose from northwestern British Columbia.

The sequences of moose from Canada that we obtained from external sources were composed of 8 haplotypes, 5 of which were identical to haplotypes described herein (A, F, J and O), and 2 others were represented in the minimum-spanning tree as presumed intermediate haplotypes (adjacent to haplotypes E and I). The 7th haplotype represented a new sequence for our network, and took a position on a new branch of length 2 from the central haplotype (A). One moose from Alberta exhibited haplotype O, which represented the only animal with a confirmed location outside of eastern United States to exhibit that haplotype.

With the exception of 1 moose specimen (probably A. latifrons) from Yukon Territory, Canada, dated at 32,250 years ago (not shown), all fossil remains identified as A. alces were deposited within the last 15,000 years; their age distribution varied geographically (Fig. 3.3). All specimens >8,000 years old occurred within the range of *A. a. gigas*. Other than 1 specimen dated at 7,848 years old within the range of *A. a. andersoni*, no specimen was older than 5,110 years for the 3 southern subspecies.

DISCUSSION

Templeton (1998) identified 3 factors that led to spatial structuring of genetic variation: 1) population fragmentation; 2) restricted gene flow, notably isolation by distance; and 3) range expansion. The star-like phylogeny of haplotypes indicated recent expansion of a single population rather than of 4 separate populations as postulated by Peterson (1955). Furthermore, we know of no historical events that could have sundered populations of North American moose for long periods after a post-ice age colonization of the continent. Therefore, we reject fragmentation as an explanation for subspeciation of moose in North America.

Isolation by distance was not detectable on a continent-wide scale in North American moose, which was consistent with a prediction of Slatkin (1993). Haplotype composition of peripheral populations of moose in North America, however, was not similar to that of populations occupying the center of the continent. That result was not expected under the Slatkin (1993) model. Therefore, we conclude that the differentiation seen in our samples was not generated by isolation by distance as a function of τ under the Slatkin (1993) model of standard stepping-stone expansion. That model assumed that

range expansion occurred as a stepping-stone progression with adequate numbers of founders for each successive population to ensure that genetic composition remained similar between adjacent populations. We hypothesize that few individuals founded peripheral populations of moose, thereby leading to the notable lack of variation in mtDNA in peripheral regions of North America.

One mechanism that may explain the phylogeographic pattern we observed is rare, long-distance (i.e., leptokurtic) dispersal (Hewitt 1996). Under that pattern, the extremely small proportion of the pre-expansion population that disperses long distances and survives, colonizes peripheral populations. Because of the distance of the dispersal, founding individuals have an opportunity to saturate the available habitat with descendants before the area is reached via normal range expansion, thereby promoting genetic homogeneity in founded populations. Simulations have determined that populations founded by rare, long-distance dispersal have lower genetic diversity compared with populations founded by a traditional stepping-stone mode (Ibrahim et al. 1996). Moreover, moose exhibit patterns of movements necessary to facilitate genetic structure among populations via rare, long distance dispersal: a high degree of female philopatry combined with the ability to disperse long distances (Hundertmark 1998).

Our genetic data are consistent with a recent colonization of North America by moose, the rapid expansion of a centrally located population, and further range expansion characterized by small numbers of founders for peripheral populations. That scenario is consistent with paleontological evidence presented herein. Rapid morphological change must have followed that colonization because paleontological evidence indicated that

moose colonizing Alaska were larger than modern moose (Guthrie 1984). Attributes for which differences exist between forest-dwelling moose of Canada and the lower United States and tundra-dwelling moose ot Alaska include body size, antler size, pelage coloration, group size, and mating behavior (Peterson 1955; Peek et al. 1974; Gasaway et al. 1987; Molvar and Bowyer 1994, Bowyer et al. 1991; Bowyer et al. 1997; Bowyer et al. in press). Those differences indicate that natural selection has acted on moose in North America since colonization, causing rapid evolutionary change. A period of rapid change combined with range expansion and restricted gene flow between source and sink populations are factors that could contribute to formation of subspecies without concomitant divergence of mtDNA lineages.

The current genetic structure of moose populations in North America is a function of movement corridors available to the colonizing population. A colonizing wave of moose entering North America through Beringia would have had limited options for dispersal to the lower portions of the continent. That dispersal would have been constrained by the presence of the Laurentide and Cordilleran ice sheets of the Wisconsinan ice age. Those ice sheets formed a barrier between eastern Beringia (Alaska) and the remainder of the continent until approximately 14,000 years ago (Dyke and Prest 1987). Subsequently, a corridor was formed along the eastern slope of the Rocky Mountains as the ice sheets retracted (Dyke and Prest 1987). Moose following that corridor would have traveled south along the eastern front of the mountains and would have exited the corridor in the area of southern Alberta, the present location of the contact zone between *A. a. andersoni* and *A. a. shirasi*. Expansion to the east likely

would have been possible until moose reached the area presently occupied by the Great Lakes. Until at least 8,000 years ago, that area was a series of large proglacial lakes (e.g., Ojibway, Agassiz, and Algonquin--Dyke and Prest 1987) that would have hindered further expansion to the east, unless moose traveled south of the lakes. Peterson (1955) reported an absence of moose in the region between the Great Lakes and Hudson Bay until recently, which would have represented a barrier to gene flow between *A. a. americana* and *A. a. andersoni*. The lack of haplotype sharing between our samples from eastern and central North America support the hypothesis that those populations have not experienced high levels of gene flow historically, and may have been separated until recently. We hypothesize that by exploiting habitat south of the lakes, moose migrated to the eastern part of the continent, and became isolated from moose in central North America as climatic warming made habitation south of the lakes unsuitable (Telfer 1984, Renecker and Hudson 1990). A similar isolating mechanism could have operated in the Rocky Mountains, restricting gene flow between *A. a. shirasi* and *A. a. andersoni*.

The eastward colonization of North America by moose at the start of the Holocene likely was facilitated by the retreating ice sheets, and subsequent growth of successional shrubs that accompanied that process–moose make extensive use of such seral stages, which provide important forages (Peek 1974, 1998). Likewise, aquatic plants, which offer a source of sodium for moose (Belovsky and Jordan 1981), probably were associated with retreating glaciers, thereby enhancing those areas for moose. Finally, the boreal forest was expanding northward following the Wisconsinan ice age, and moose would have encountered productive habitats, especially areas undergoing

succession following fire (Peek et al. 1976; Loranger et al. 1991; Weixelman et al. 1998). Those successional stages undoubtedly hastened the eastward expansion by moose, and offer an ecological mechanism for the genetic patterns we documented.

The low mitochondrial diversity of moose from mainland Alaska was unexpected, and contradicts the relatively high allozyme diversity observed by Hundertmark et al. (1992) in those moose. That region was the first area of North America to be colonized by moose (Guthrie 1995, Chapter 2), we expected to find high diversity there based on our explanation for the colonization process. A decrease in effective population size at some time since colonization (i.e., a population bottleneck) would explain the low mitochondrial diversity, yet may be consistent with observations of allozyme diversity, because the effective size of the nuclear genome is 4 times the size of the mitochondrial genome. As such, allozymes diversity is less susceptible to bottlenecks.

The only region of North America that was not represented well in our samples was the Rocky Mountains of Canada. Nonetheless, data concerning moose inhabiting that area that we obtained from other studies (Mikko and Andersson 1995, Polzeihn and Strobeck 1998) indicated that our sampling encompassed much of the variation present in North America. Furthermore, the high haplotype diversity represented by those data indicated that the Rocky Mountains region of southern Canada had a level of diversity similar to that observed in British Columbia and central North America. That outcome further supports our contention that *A. a. andersoni* represents a diverse set of haplotypes and likely retained a relatively high effective population size during colonization.

Taxonomic implication

Designation of subspecies should rely on analysis of multiple criteria rather than merely on analysis of genetic data (Cronin 1993). Yet, mtDNA is an informative locus for intraspecific phylogeography (Avise et al. 1987) and can provide insights into population history. Our data indicate a pattern of genetic structure among regional moose populations caused by lack of gene flow, and are consistent with some degree of isolation of populations in the past. Moreover, those data are consistent with morphological characteristics used by Peterson (1955) to describe subspecies of moose in North America. Evidence from other taxa has indicated that small effective sizes of populations established in a similar manner have led to extensive and rapid differentiation (Hewitt 1996). Although further investigations incorporating nuclear loci, particularly in the regions of contact zones, may be necessary to achieve a final conclusion, there is evidence of reproductive isolation among regional populations of moose in the past, which may have caused enough divergence to support subspecies status.

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Table 3.1. Variable nucleotide sites within the left hypervariable domain of the mitochondrial control region in North American moose (*Alces alces*). The nucleotide positions are numbered relative to the first nucleotide of the control region sequence and consist of 470 sites. The Alaska1 haplotype is listed as the reference and variable sites in other haplotypes are indicated. Identical sites are indicated by a dot. The numbers of each haplotype occurring in the populations sampled are indicated in columns at the right.

		Population					
Haplotype	1122222233333334444 8160225880012246022 5 6082487120381675089 5	Alaska	Southeastern Alaska	BC	Colorado	Central	East
A	TCGTCTCATAACTTTCAGCC	21				1	
B	.............C......	39		1			
C	....T.TGC.......G...	1	12	1			
D	....T..GC.......G...		1				
E	...C....C...C.......			6			
F	C.....T....T......T.			1			
G	..A.T.TGC.......G...			1			
H	...C..T.......C.....			1			
I	......T.....C......T				19		

Table 3.1 (continued)

J	..............T.A..					6	
K	........CGG.....					1	
L	..............T....					9	
M	.T..........T....					1	
N	C.........T..C...T.					6	
O	.....C.....						12
P	...C.C.....						1
Total		61	13	11	19	23	13

Table 3.2. Parameters indicating intrapopulation-level diversity of mtDNA for North American populations of moose (*Alces alces*). Subspecific designations for populations follow Hall (1981). Values in parentheses equal variances of the estimates.

Population (subspecies)	N	No. haplotypes/ no. private haplotypes	Haplotype diversity (*H*)	Nucleotide diversity (π)	Segregating sites (*S*)
Alaska (*A. a. gigas*)	61	3/1	0.49 (0.03)	0.0013 (0.0009)	6
Southeastern Alaska (Indeterminate)	13	2/1	0.15 (0.12)	0.0011 (0.001)	1
Colorado (*A. a. shirasi*)	19	1/1	0	0	0
Western (*A. a. andersoni*)	34	11/9	0.86 (0.03)	0.0072 (0.004)	17
Central	24	6/5	0.76 (0.05)	0.0052 (0.003)	10
British Columbia	11	6/4	0.73 (0.14)	0.0087 (0.005)	13
Eastern (*A. a. americana*)	13	2/1	0.15 (0.13)	0.00033 (0.0005)	1

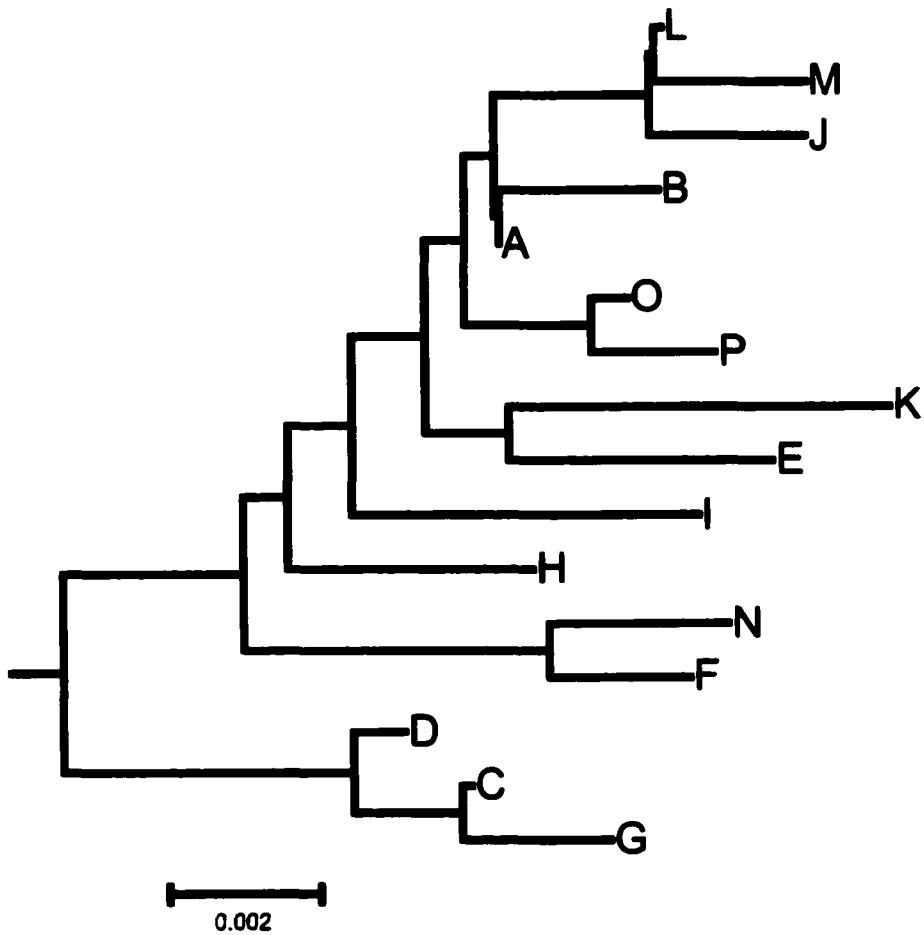

Fig. 3.1. A neighbor-joining phylogeny of the control region for North American moose (*Alces alces*). This tree was rooted with the homologous sequence from a caribou (*Rangifer tarandus*), but that branch is not shown.

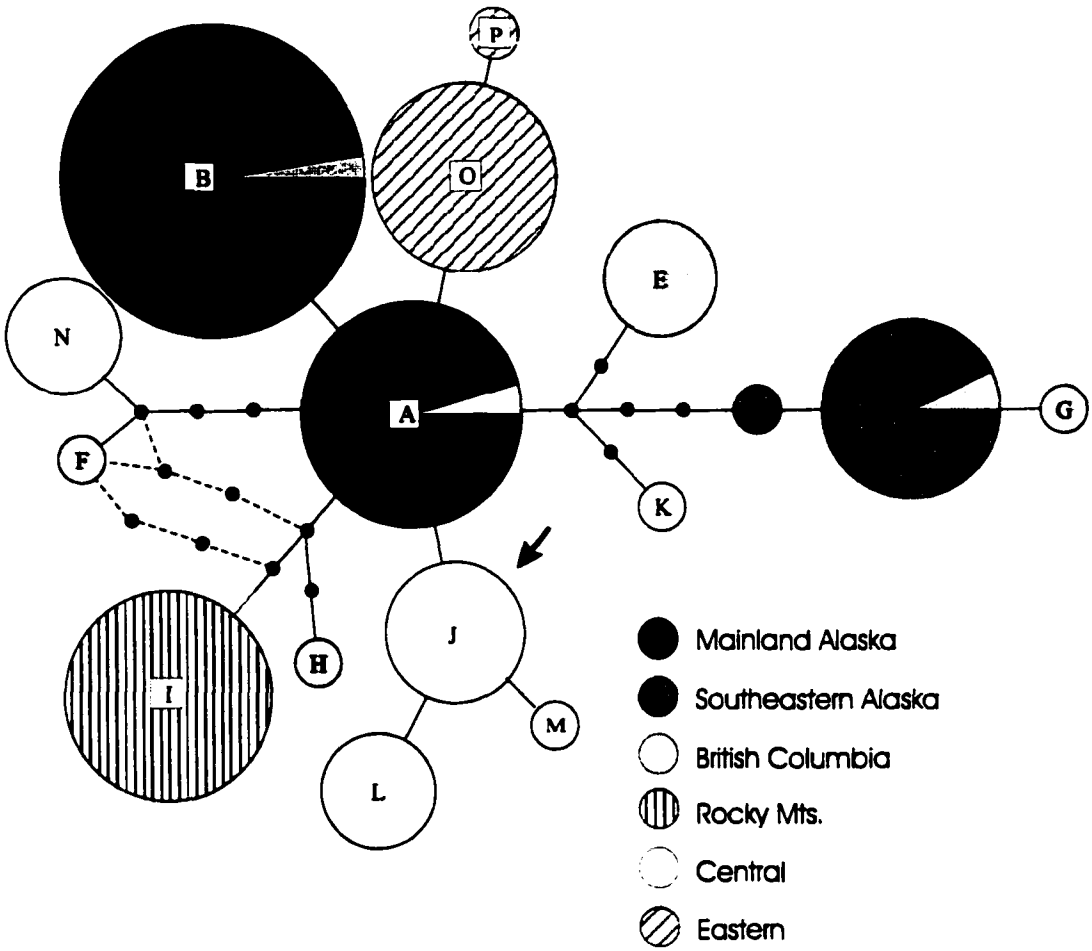

Fig. 3.2. Minimum spanning tree of mitochondrial haplotypes of North American moose (*Alces alces*). Labeled circles indicate haplotypes identified in this study shown in Table 3.1, and dots indicate presumed intermediate haplotypes based on a most-parsimonious arrangement of mutations. Alternate equally parsimonious pathways are indicated by broken lines. Size of circles is proportional to occurrence of haplotypes, and partitions within circles indicate geographic distribution of haplotypes.

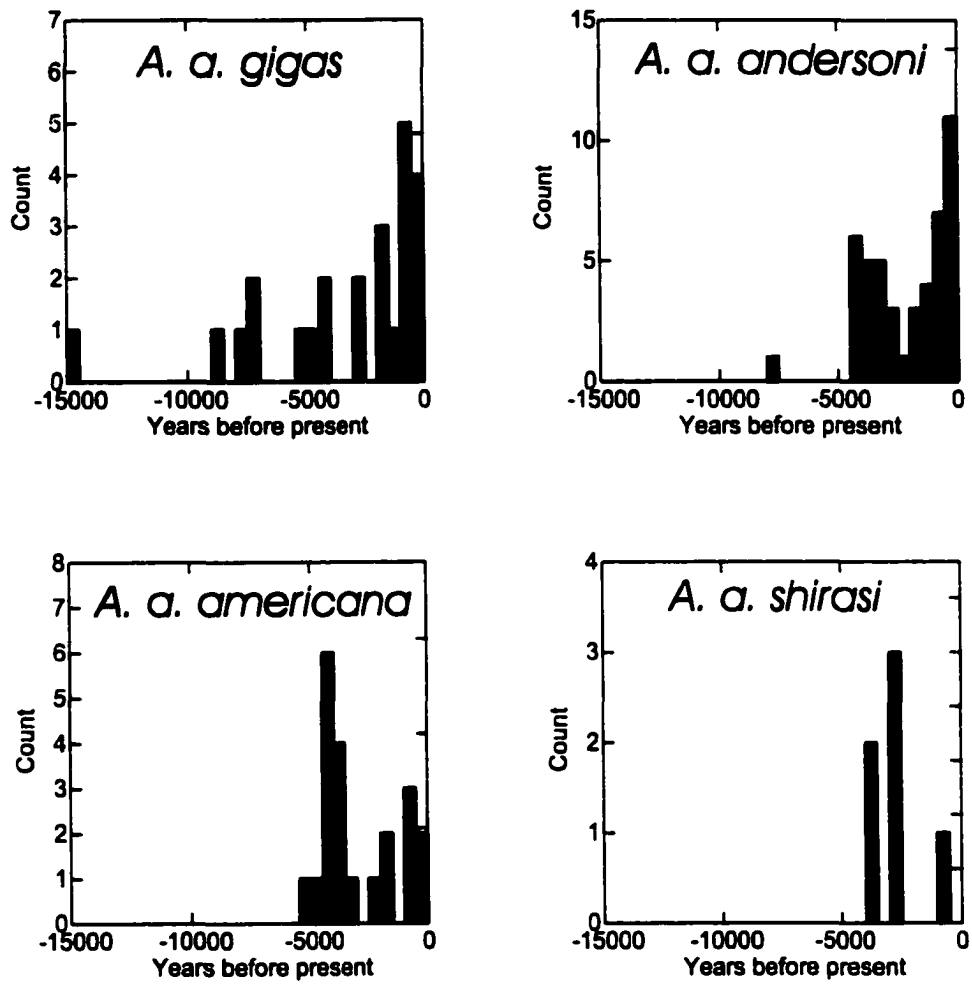

Fig. 3.3. Distributions of radiometric dates for fossil remains of moose (*Alces alces*) within the approximate ranges of North American subspecies. One specimen with a date of 32,250 years ago within the range of *A. a. gigas* is not shown.

CHAPTER 4[4]

SPATIAL VARIATION IN mtDNA HAPLOTYPES FROM MOOSE IN ALASKA AND NORTHWESTERN BRITISH COLUMBIA: TAXONOMIC AND MANAGEMENT IMPLICATIONS

Abstract We assessed phylogeographic history of moose (*Alces alces*) in southeastern Alaska, USA, by determining their genetic affinity to surrounding populations thereby clarifying their origin and uncertain taxonomic status. Moose from central and southern regions of the southeastern Alaska panhandle were characterized by two haplotypes that were highly divergent from those in the remainder of the state; overlap occurred only in the northernmost area of the panhandle. Moose inhabiting British Columbia, Canada, showed high haplotype diversity, which they shared with moose in southeastern and interior Alaska, in addition to having haplotypes that were restricted to that area. Similarity between geographic distribution and phylogenetic structure of haplotypes indicates temporal and spatial separation of moose in the past. Moose representing an early split from the colonizing wave in the late Pleistocene and early Holocene may have colonized major river drainages of southeastern Alaska. Other lineages in British Columbia likely belong to a subsequent invasion of moose colonizing from the southeast. Coastal populations of moose living south of 58° 45' N latitude in southeastern Alaska should not be classified as *A. a. gigas*. Behavioral and morphological differences

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between *A. a. gigas* and other forest-dwelling subspecies in North America indicate a need to reevaluate management practices in southeastern Alaska.

INTRODUCTION

The zone of intergradation between the Alaskan moose (*A. a. gigas*) and the northwestern moose (*A. a. andersoni*) occurs in central Yukon Territory (Peterson 1955, Gauthier and Larsen 1985), but the location of that zone where it intersects the coast of the Gulf of Alaska, and therefore the subspecific identity of moose in that area, is uncertain. Klein (1965) suggested that moose arrived recently in southeastern Alaska, USA, by migrating down major river corridors draining northwestern British Columbia, Canada, through the coastal mountains that form the international boundary. He identified those moose as *A. a. andersoni*, based on the supposition that they represented a population expansion of that subspecies from interior British Columbia. Conversely, Hall (1981, p. 1101) indicated that moose in the northern one-half of the southeastern panhandle of Alaska are *A. a. gigas*, and that moose do not occur in the southern portion of the panhandle or in neighboring areas of British Columbia to the east and south.

Irrespective of which race of moose occurs in southeastern Alaska, these large mammals are important for recreational and subsistence hunting (Timmermann and Buss 1998), as well as a valuable resource for the tourism industry (Snepenger and Bowyer 1990). Management objectives for moose in Alaska generally are formulated at the level of the game management unit or subunit, which are areas defined by geographical criteria. Management objectives can differ among units, but generally follow the same guidelines. An important parameter in moose management is the ratio of adult males to

100 adult females (bull-cow ratio). That ratio is determined during aerial censuses in late autumn and includes only moose >1 year of age, and therefore is considered as an index to the likelihood of a female conceiving in her first estrus (Timmermann and Buss 1998).

Objective levels for bull-cow ratios in Alaska are set under the assumption that moose possess a harem mating system (Schwartz 1998). Under that system, one bull assembles a harem of females and monitors them closely for signs of estrus. That mating structure has evolved in animals inhabiting open habitat (Bubenik 1987; Molvar and Bowyer 1994), and bull-cow ratios as low as 20-30:100 are thought sufficient to ensure proper synchrony of rut (Schwartz 1998). Moose in the remainder of North America exhibit a tending-bond mating system (sensu Hirth 1977), wherein a bull courts one female and remains with her until she comes into estrus (Bubenik 1987; Bowyer et al. in press). After mating with her, the bull seeks out another female (Bubenik 1987). That mating system evolved in forested habitat, and management guidelines in many jurisdictions in Canada require bull-cow ratios >30:100 and often >50:100 (Crête et al. 1981; Timmermann 1992). Synchrony of rut helps ensure that females are bred during their first estrus (Schwartz and Hundertmark 1993, Bowyer et al. 1994, Whittle et al. 2000). Young conceived during the second estrus do not achieve maximum size by their first winter (Schwartz et al. 1994; Keech et al. 1999), and later-born offspring have lower overall survival rates than offspring born earlier (Keech et al. 2000). A positive relationship was observed between size of harvested calves and bull-cow ratio in autumn for populations of moose in Quebec, Canada (Taquet et al. 1999).

The mating behavior of moose in southeastern Alaska is undocumented, primarily because habitat in that area is characterized by thick forests (Doerr 1983) and frequent inclement weather, which preclude routine observations from the air. Access along the ground is limited because most populations occur in roadless areas, although waterways provide limited access with shallow-running watercraft. Populations of moose are small and isolated, and research has focused on habitat use and effects of commercial forestry on moose (Doerr 1983, Hundertmark et al. 1990), rather than mating behavior.

Clarification of relationships of moose in southeastern Alaska with those of *A. a. gigas* and *A. a. andersoni* via a phylogeographic assessment of population history is an indirect but feasible method to gain insight into their life-history strategies, and facilitate creation of appropriate management objectives. We tested the hypothesis that moose in southeastern Alaska would show a closer phylogenetic relationship to *A. a. gigas* than to *A. a. andersoni* of northwestern British Columbia.

MATERIALS AND METHODS

We analyzed a subset of genetic sequences described in Chapter 3, selecting those sequences from individuals collected within Alaska and British Columbia. We defined populations based on geographic and political boundaries. All samples collected within the province of British Columbia were considered as one population. Moose from Alaska were divided into two populations, one consisting of moose collected in the southeastern panhandle, and the other representing those from the remainder of the state. The boundary between those areas was Glacier Bay National Park and Preserve (58°30'N, 136°W). Specimens were obtained from each of the major river drainages in

southeastern Alaska and represented all known populations of moose in the region that were established naturally. Exceptions were populations in Berners Bay (58°45'N, 135°W) and the Chickamin River (55°50'N, 131°W), which were founded by animals introduced from other areas of Alaska (Burris and McKnight 1973); moose from those populations were not included in our analysis.

Detailed descriptions of the amplification and sequencing processes for mtDNA were provided in Chapter 3. The targeted sequence within the mtDNA molecule was the left hypervariable domain of the control region. That portion of the mitochondrial genome evolves at an extremely fast rate and is useful for constructing phylogenies in cervids (Douzery and Randi, 1997), particularly among recently diverged taxa, as well as for studies of intraspecific differences among populations. Primers LGL283 (L15927, 5'-TACACTGGTCTTGTAAAC-3') and ISM015 (H00171, 5'-ATGGCCCTGTAGAAAGAAC-3') were used to amplify the control-region sequence. Unlike the sequences analyzed in Chapter 3, the sequences analyzed herein also included the noncoding strand of the $tRNA^{Pro}$ gene and a portion of the $tRNA^{Thr}$ gene.

Phylogenetic relationships among sequences were assessed with a neighbor-joining tree (Saitou and Nei 1987) with program PHYLIP (Felsenstein 1993), constructed with the two-parameter distance estimate of Kimura (1980). Estimates of intra- and inter-population level variability were computed with the software ARLEQUIN (Schneider et al., 1997). Intra-population level variation was expressed as haplotype diversity (H) and nucleotide diversity (π) (Nei 1987). Inter-population variation was

expressed as pairwise Φ_{ST}, which is a molecular equivalent of Wright's F_{ST} (Excoffier et al. 1992).

RESULTS

Eight haplotypes were identified among 87 moose (Table 4.1). All substitutions were transitions and were located in the control region. Sequences were organized into three motifs (Fig. 4.1) that were clustered geographically (Fig. 4.2). Two haplotypes (Alaska1 and Alaska2) were distributed in the main portion of Alaska and those differed from each other by one substitution ($\pi = 0.09\%$, $H = 0.49$). Both haplotypes were found north of the Alaska Range, which separates the interior and northern portions of the state from southcentral Alaska. Only 1 haplotype (Alaska2) was found south of the Alaska Range. Moose in southeastern Alaska were characterized by three haplotypes ($\pi = 0.54\%$, $H = 0.55$); two of those (SEAlaska1 and 2) differed by only one substitution and were highly divergent from the third haplotype (Alaska2), which was a type common to interior Alaska and occurred only in the northernmost section of the panhandle. In British Columbia, six haplotypes were documented among 11 individuals ($\pi = 0.74\%$, $H = 0.73$). Those included one moose from the northern part of the sampling area with the Alaska2 haplotype and one moose in the central portion of our sampling area with the SEAlaska1 haplotype. Of the four haplotypes unique to British Columbia, one was closely related to a southeastern Alaska haplotype, differing by one substitution. Otherwise, the three other haplotypes of moose from British Columbia were not closely related to any Alaskan haplotypes, and were distributed in the southern portion of our sampling area in British Columbia (Fig. 4.2). Pairwise Φ_{ST}s between populations were

0.67 between Alaska and British Columbia, 0.59 between Alaska and southeastern Alaska, and 0.27 between southeastern Alaska and British Columbia, indicating the closer relationship between southeastern Alaska and British Columbia than between either of those populations and Alaska.

DISCUSSION

Concordance of geographic distribution and phylogenetic structure of haplotypes in our sample is indicative of spatial and temporal separation of populations. Such a separation is necessary for the establishment of differences that define subspecies. Our data indicate that there are three separate populations partitioned into two putative subspecies. The pairwise Φ_{ST}s indicate a greater level of gene flow between moose in southeastern Alaska and those in British Columbia than between either of those populations and moose in the main part of Alaska.

Distribution of haplotypes in Alaska showed a geographic structure related to glacial history. The southeastern Alaska-British Columbia area was covered by the Cordilleran ice sheet during the Wisconsinan glaciation (Fulton et al. 1986). That ice sheet extended to the east as far as the Rocky Mountains and during glacial retreat served as the western boundary of an ice-free corridor that may have served as a movement corridor for moose as they colonized central North America (Geist 1987, Cronin 1990, Bowyer et al. 1991, Hundertmark et al. 1992, Chapter 3). The Cordilleran ice sheet also covered southcentral Alaska south of the Alaska Range, but much of the land north of the Alaska Range was ice-free. It is likely that moose colonized habitat north of the Alaska Range during the colonization of the continent and followed that habitat to the corridor

leading south. Subsequently, dispersing individuals eventually found suitable habitat in previously glaciated areas.

Moose in southeastern Alaska either diverged early from the first colonizers of the continent and migrated up the coast, or they arrived via a general movement of moose down the major river corridors from interior British Columbia. Topography of the coast in southeastern Alaska is rugged and dominated by a coastal rain forest of spruce-hemlock (*Picea sitchensis-Tsuga heterophylla*), which is not productive habitat for moose (Telfer 1984). Successful dispersal along that coast is unlikely, except to areas adjacent to major river drainages such as Thomas Bay, which is 34 km to the northwest of the mouth of the Stikine River (Fig. 4.1). Populations in southeastern Alaska, therefore, must have arisen through recent migration down the river valleys from populations in British Columbia, and this only recently, supporting the contention of Klein (1965). Peterson (1955) summarized reports from the late 19th and early 20th centuries that indicated a range expansion of moose from extreme northeastern British Columbia toward the coast, which may refer to moose that eventually colonized southeastern Alaska. The 1 exception to this scenario is the population inhabiting the Chilkat River valley in northernmost southeastern Alaska. Moose dispersing into that valley likely originated in the boreal forest region of southwestern Yukon Territory, which is contiguous with the distribution of the Alaskan-like sequences, and the designation of *A. a. gigas*. The lack of variability within populations of moose in southeastern Alaska and the low proportion of southeastern Alaska haplotypes in interior British Columbia is intriguing. Whatever the actual scenario, we believe that the lineage

of moose in southeastern Alaska is the remainder of a relict group of moose that split very early from the main colonizing wave. Those animals may have been present in the area in very small numbers because of limited habitat availability, or may have recently moved down the major rivers to colonize this area (Klein 1965).

Moose inhabiting the interior portions of northwestern British Columbia exhibited haplotypes that resembled those from coastal Alaska and central North America (Chapter 3). Peterson (1955) reported that moose from the area of the Montana-British Columbia border expanded their range to include southern and central British Columbia early in the 20th century, perhaps aided in their movements by anthropogenic influences on the landscape such as road building and commercial logging. The distribution of haplotypes in British Columbia, therefore, is a result of admixture that occurred between two populations.

We suggest that southeastern Alaska contains two subspecies of moose. Moose north of the latitude of Berners Bay (58° 45' N latitude, Fig. 4.1), which are classified as *A. a. gigas* based on the common lineage they share with moose inhabiting areas to the north. Although we did not have access to samples from Yukon Territory, we can speculate that the boundary extends through central Yukon as depicted by Hall (1981). Moose inhabiting the major river drainages of southeastern Alaska south of Berners Bay are most appropriately considered a separate subspecies. Moose populations in the northern part of the panhandle of Alaska and in extreme northwestern British Columbia carry haplotypes that occurred in both groups and undoubtedly represent a transition zone. A conservative approach to taxonomy would classify moose in central and

southern southeastern Alaska as *A. a. andersoni*. That classification could be justified on the assumption of substantive gene flow along the river valleys between the moose from southeastern Alaska and British Columbia. Species with strong female philopatry, such as moose (Hundertmark 1998) or white-tailed deer (Purdue et al. 2000), can experience nuclear gene flow through male dispersal without changes in the structure of mtDNA in populations (Paetkau et al. 1998). The apparent geographic separation of three lineages in our study area, however, indicates that the lineages were effectively isolated at some time in the past, which may have resulted in divergence justifying separate taxonomic status of moose in southeastern Alaska. Nonetheless, the close affinity between moose in southeastern Alaska and northwestern British Columbia indicates that moose in southeastern Alaska likely share more traits with populations to the east than with populations to the north. Moreover, the potential for gene flow along the coast is much less than the potential for gene flow along river valleys, which would act as a disruptive force against maintaining the coastal populations as a separate taxonomic unit.

Taxonomic units are not normally based solely on genetic differences but usually depend primarily on morphological characteristics in the Cervidae. Although subspecies of deer have at times been shown to diverge genetically (Smith et al., 1986), other studies frequently fail to show that they do (Hillestad 1984, Cronin, 1991, Ellsworth et al. 1994a and 1994b). Ellsworth et al. (1994a) describes one such divergence that does not co-occur with the recognized subspecific ranges, which he interprets in terms of dispersal routes created by low sea levels along the coastal plain of the southeastern United States during glacial periods. Sometimes the genetic differences may indicate specific rather

than subspecific status of the forms involved, such as the South American subspecies of white-tailed deer in Surinam studied by Smith et al. (1986) which are more likely to be a species like those described by Molina and Molinari (1999). Local populations show strong spatial heterogeneity and often do not share mtDNA haplotypes in white-tailed deer over even limited geographical areas along the southeastern coastal (Purdue et al. 2000). Given the common occurrence of spatial heterogeneity in genetic characteristics in deer, care is needed when using only genetic differences to designate taxonomic forms, and it would be much better if the designation also depended upon other differences. The two recommended subspecies of moose not only differ genetically but in other characteristics as well.

A close relationship between moose from southeastern Alaska and northwestern British Columbia may indicate that moose from southeastern Alaska should be managed as if they were *A. a. andersoni*. Objectives for bull-cow ratios in those populations should be increased to reflect the tending bond mating system of *A. a. andersoni*. Because antler size of *A. a. andersoni* is smaller than that of *A. a. gigas* (Gauthier and Larsen 1985, Gasaway et al. 1987, Bowyer et al. in review), current harvest restrictions in southeastern Alaska based on antler size and conformation should be reconsidered. Current regulations that define one group of legal animals as having antlers ≥127 cm in width fortuitously may allow a rapid rise in bull-cow ratios, because relatively few bulls likely would be harvested based on that criterion.

We documented a range extension of moose in northwestern British Columbia and southeastern Alaska. We obtained tissue samples from moose as far south as the

Skeena and Endako rivers in British Columbia (approximately 54° N latitude), as well as the Unuk and Stikine rivers in southeastern Alaska. Those areas were not included within the range of moose by Hall (1981) or more recently by Karns (1998). Although moose likely have been present in lower southeastern Alaska for >100 years (Klein 1965), those large cervids may have colonized the Skeena region of British Columbia relatively recently.

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Table 4.1. Distribution of the number of control region haplotypes in moose populations in Alaska and northwestern British Columbia (Fig. 4.1), and matrix of variable nucleotide sites. Numbering of sites refers to position relative to the first nucleotide at the 5' end of the sequence. Dots in the nucleotide matrix reflect identity with the first sequence. C, A, T, and G refer to nucleotide bases cytosine, adenine, thymine, and guanine, respectively.

	Location				Nucleotide position												
Haplotype	Alaska	Southeastern Alaska	British Columbia	Total	170	252	286	308	341	365	366	402	405	410	431	484	513
Alaska1	21			21	T	G	T	C	C	A	T	C	T	T	T	A	C
Alaska2	32	9	1	42	•	•	•	•	•	•	•	•	•	C	•	•	•
SEAlaska1		13	1	14	•	•	•	T	T	G	C	•	•	•	•	G	•
SEAlaska2		1		1	•	•	•	T	•	G	C	•	•	•	•	G	•
BC1			6	6	•	•	C	•	•	•	C	•	C	•	•	•	•
BC2			1	1	C	•	•	•	T	•	•	T	•	•	•	•	T
BC3			1	1	•	A	•	T	T	G	C	•	•	•	•	G	•

Table 4.1 (continued).

BC4			1	1	•	•	C	•	T	•	•	•	•	•	C	•	•
Total	53	23	11	87														

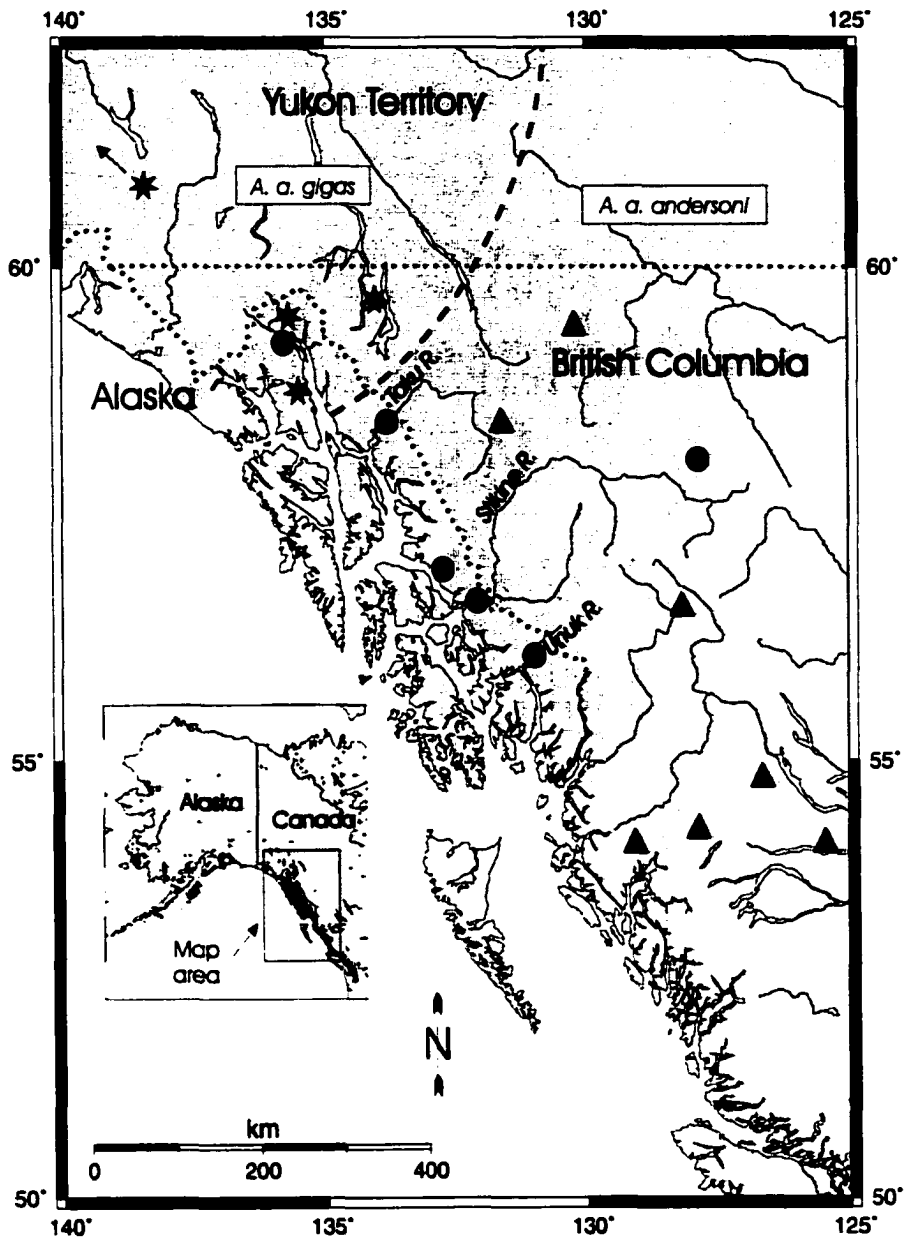

Fig. 4.1. Map of southeastern Alaska and adjacent areas of Canada. Symbols represent occurrence of at least one haplotype of a given group. Stars represent the Alaska haplotypes, circles represent southeastern Alaska haplotypes, and triangles represent British Columbia haplotypes not closely related to those in Alaska. The star and arrow in

the upper left indicate that all haplotypes from the remainder of Alaska were the Alaska motif. The dashed line is our proposed boundary between *Alces alces gigas* and *A. a. andersoni*; the orientation of the boundary in Yukon Territory is speculative and follows Hall (1981).

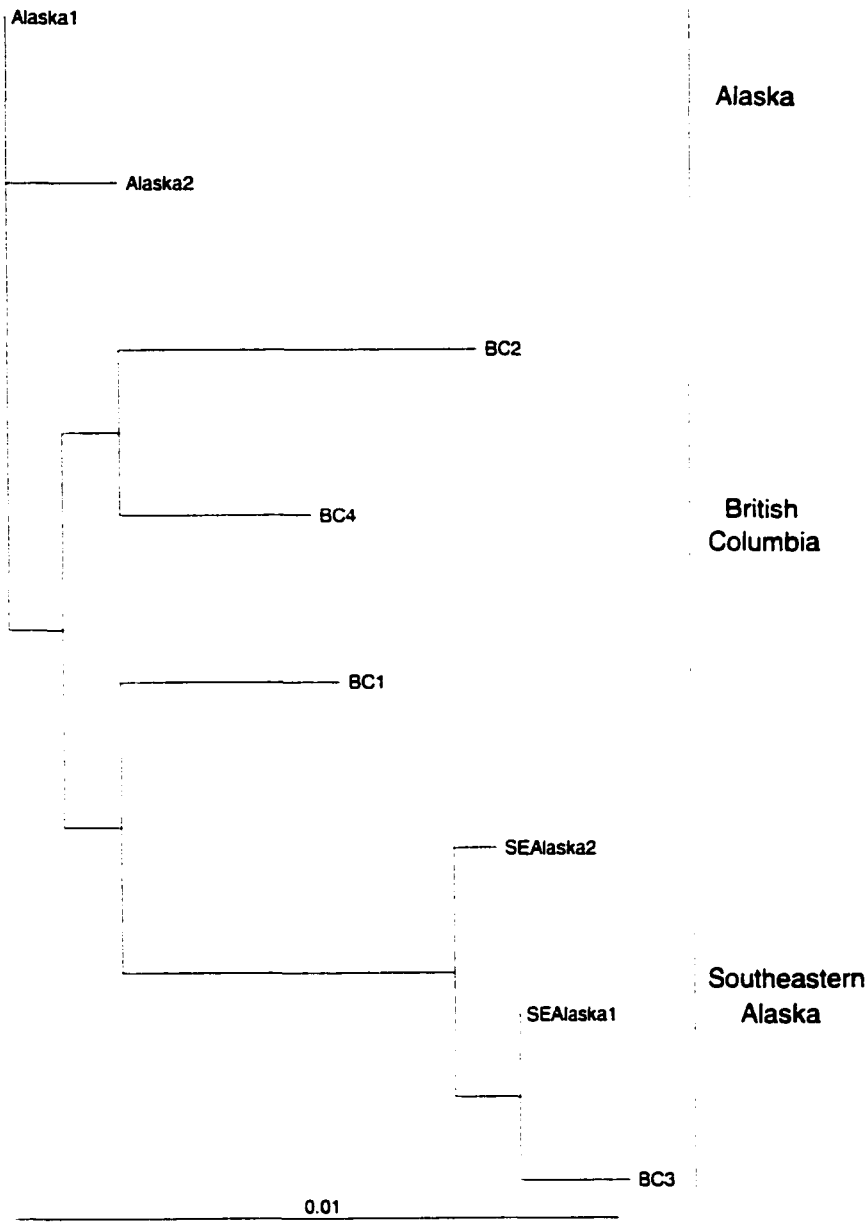

Fig. 4.2. Unrooted neighbor-joining tree of mitochondrial control region haplotypes found in Alaska and British Columbia. The areas in which the three major sequence motifs occurred are indicated at the right.

SYNOPSIS AND CONCLUSIONS

Phylogenetic relationships among mitochondrial haplotypes of moose worldwide indicated that Asia likely served as the source for all extant lineages. Asian lineages were documented in all clades of phylogenetic trees, but European and North American lineages were confined to separate clades, indicating their more recent divergence. Those relationships did not support the existence of phylogenetically distinct eastern and western races of moose. North American lineages were more closely related to European lineages than either was to Asian lineages. Moreover, I documented that a length mutation in the mitochondrial control region that heretofore had been viewed as indicative of such a systematic division was not related to such a geographic split.

Indices of nucleotide and haplotype diversity of mtDNA within and between populations of moose were low for a species with a circumboreal distribution. Low diversity on such a large scale indicates an historic bottleneck followed by population and range expansion. Analysis of mismatch distributions indicated that the expansion was sudden in most instances, and that 2 expansions occurred at the end of the Pleistocene. Those expansions were contemporaneous with periods of climatic warming following glacial periods. Climatic warming undoubtedly caused proliferation and northward expansion of boreal-forest communities to which moose are currently adapted. My data support the contention of Guthrie (1995) that the expansion of boreal forest after the most recent glacial maximum was the mechanism by which moose entered North America through Beringia.

The star-like phylogeny of North American moose indicated a recent and sudden expansion. Considering that phylogeny contained haplotypes from central Asia, but not eastern Asia, and that haplotypes in eastern Asia were not closely related to those in North America, I concluded that moose currently inhabiting areas on either side of the Bering Strait were not closely related. Those lineages in eastern Asia do not represent lineages that were involved in the northward expansion that ultimately resulted in the colonization of North America. Rather, those lineages must represent a subsequent colonization of northeastern Asia following local extirpation. Low diversity of moose in Alaska compared with populations to the east also supports an hypothesis of near extirpation and recolonization of the northernmost areas of the current range of moose.

The pattern of genetic variation observed in North America indicated a process of colonization consistent with a central population of large effective size providing small numbers of founders for peripheral populations. My observations are consistent with a model of range expansion by leptokurtic dispersal after initial colonization. Moreover, selection pressure on a colonizing moose population favoring hypomorphy, combined with a colonization process compatible with isolation of regional populations, may explain the rapid divergence of 4 North American subspecies. Range expansion by leptokurtic dispersal has been proposed as a mechanism behind rapid intraspecific divergence in other taxa (Hewitt 1996).

The divergence and expansion of moose populations in the late Pleistocene resembled that for humans. Similarities include region of origin for North American migrants, timing of the colonization, and indications of a post-colonization bottleneck in

Beringia. As the only 2 species of large mammals to cross from west to east across the Bering land bridge during its last appearance, those similarities indicate that common mechanisms underlay those events, and that moose may have played an important role in effecting the colonization of North America by humans.

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APPENDIX A[5]

EFFECTS OF POPULATION DENSITY AND SELECTIVE HARVEST ON ANTLER PHENOTYPE IN SIMULATED MOOSE POPULATIONS

ABSTRACT: We simulated moose (*Alces alces*) populations held either at or below carrying capacity (*K*) to determine the effect of population density on harvest rate and frequency of alleles favoring antler growth under a system of selective harvest. A stochastic model of density-dependent population growth was created to achieve stable populations at *K* with no hunting. Rates of mortality not associated with hunting were increased to simulate predation losses for a population held below *K*. The increased nutrition available to this lower-density population was assumed to result in larger age-specific antler size. Each population was subjected to a harvest plan that defined legal bulls as those with either a spike-fork antler as yearlings (small bulls) or with an antler spread of ≥50 inches (127 cm) as large bulls. Harvest, population composition, and frequency of alleles favorable to antler growth were monitored throughout the simulations. For the population held at K the frequency of favorable antler alleles declined slightly from that obtained in the population with no hunting. When the population was reduced below *K*, harvest decreased and the proportion of small bulls in the harvest increased compared with the population at *K*. In the population below *K*, the frequency of favorable alleles declined steadily, likely to fixation for unfavorable alleles.

[5] Hundertmark, K. J., T. H. Thelen, and R. T. Bowyer. 1998. Effects of population density and selective harvest on antler phenotype in simulated moose populations. Alces 34:375-383.

Ratios of bulls:100 cows in the two harvested populations were similar but ratios of small:large bulls were changing, with the population at lower density exhibiting a higher proportion of small bulls prior to harvest. Under the conditions imposed by our model, increases in age-specific antler size associated with increased nutrition resulted in greater selection against alleles favorable for antler growth under a scenario of selective harvest. Changes in density of moose populations and resulting effects of nutrition on the potential for antler growth must be considered when predicting the outcome of antler-based selective harvests.

INTRODUCTION

Selective harvest of moose based upon antler size is a common management practice in Alaska and has been an effective management tool (Schwartz et al. 1992). This strategy permits harvest of bulls with either a spike or forked antler (hereafter referred to as small bulls) or having an antler spread of at least 127 cm (50 in; large bulls). Any bull having at least 3 tines on 1 brow palm also is legal to harvest. Such a harvest plan allows a moderate level of harvest while ensuring stability in the proportion of males in the population, and a greater mean age among males than does a plan in which any bull is legal to harvest. A modeling exercise demonstrated that this harvest plan also maintained allelic diversity among hypothetical loci coding for antler growth with the exception of alleles coding for numerous brow tines (Hundertmark et al. 1993).

The effect of environment on antler growth can be considerable, with estimates of up to 50% of variability in antler size attributable to the environment (Harmel 1983). At population densities below nutritional carrying capacity (K), cervids should exhibit larger

age-specific body and antler size because of greater availability of nutritious forage to individuals (McCullough 1984). As the rate of antler growth changes in populations experiencing increasing nutrition, we hypothesize that the response of these populations to selective harvest also may change. Some managed populations of moose in Alaska are held near *K* because of hunting policies restricting harvest to males, and the population and genetic effects of selective harvest were evaluated only for populations at or near *K* (Hundertmark et al. 1993). Some populations in interior Alaska, however, are held at densities far below *K* because of predation (Gasaway et al. 1983, 1992). We conducted a modeling exercise to determine changes in genetic composition and harvest levels in a moose population held below *K* and compared our results with those from the model of populations at or near *K*.

METHODS

A stochastic population model (Fig. A.1) reported originally by Thelen (1991) and modified by Hundertmark et al. (1993) was used to simulate harvests of different populations of moose at varying densities. The model simulated populations through annual cycles of births, summer mortality of calves, harvest, mating, and winter mortality of adults and calves. All adult mortality was registered in winter. Each animal in the population was characterized by age, sex, an antler genotype and phenotype.

Antler growth was assumed to be an age-dependent polygenic trait. Antler size conforms to a polygenic model because of the continuous variation seen within age classes (Futuyma 1986). In the model, 5 pairs of genes and environmental influences were assumed to contribute to a growth, or phenotype, score for antlers (SCORE). For

each locus, there were two possible alleles: favorable and unfavorable, which contributed 4 and 0 points to the score of the genotype, respectively. Thus, the score for antler growth varied from 0-40 (allele score x two alleles/locus x five loci). The model tracked the frequency of favorable antler alleles (Q_A). Environmental scores were generated randomly from a distribution with the same mean and variance as the scores for genotypes and one score was permanently assigned at birth to each male. The sum of an individual's genotype and environmental scores created its antler phenotypic score that determined age-specific antler size. We assumed a heritability of 0.5, which meant that the genotype and environmental scores were weighted equally. Williams et al. (1994) reported mean estimates of heritability between 0.42-0.47 for antler spread, main beam length, and number of antler points for white-tailed deer (*Odocoileus virginianus*). Unlike the prior exercise (Hundertmark et al. 1993) this model did not include an option to kill a bull legally if it had at least 3 brow tines on one antler. The hypothetical locus controlling expression of brow tines was considered independent of loci encoding for antler spread and we assumed that any effects attributable to genotype at that locus would be identical for either model.

Slower rates of antler growth in yearlings were expressed as spike-fork antlers. Antlers of this size were assumed to be present only in yearlings, and accounted for 60% of antlers in that age class in a population at or near K (Schwartz et al. 1992). All other bulls had palmated antlers that were characterized by a measurement of spread. Age-dependent antler spreads (Table A.1) were assigned to the initial population based upon

data from hunter check stations on the Kenai Peninsula. Maximum spreads occurred in animals 8-12 yrs old (Gasaway et al. 1987).

To simulate the effect of increased nutrition on antler phenotype resulting from the better nutrition available to a population below *K* (sensu McCullough 1979), we multiplied the antler score (phenotype score) by a variable that changed with population size. With a population $\leq$4,000 animals, antler scores were multiplied by 1.36. This value declined exponentially until it equaled 1.00 at a population of 10,000 moose (*K*). In this way, changes in environment caused changes in phenotype yet heritability remained at 0.5 throughout the simulation. The variation produced by this function was similar to the amount of variation observed in mean antler size among populations of moose in Alaska (Gasaway et al. 1987).

The initial population was created using estimates of age structure from a population from the northern Kenai Peninsula, Alaska (Schwartz et al. 1992). Individuals in the initial population were randomly assigned genotype scores. Survival rates of females were based upon those reported for the northern Kenai Peninsula by Bangs et al. (1989) but were adjusted slightly to produce a stationary population. Survival rates of calves in summer and winter were 0.55 and 0.40, respectively. Annual rates of survival of females older than calves were 0.88 (yearling), 0.95 (2-5 yrs), 0.90 (6-10 yrs), 0.85(11-12 yrs), 0.80 (13-14 yrs), 0.70 (15-16 yrs), 0.60 (17 yrs), 0.45 (18 yrs), 0.25 (19 yrs), and 0.0 (20 yrs). Determination of individual survival was a stochastic process involving comparison of a randomly-generated number with the appropriate survival rate. Survival rates for males were based upon those of females but were reduced by an exponential

decay function in which antler-size-dependent survival (ASDS) for bulls decreased as it aged and its antler size increased. The function determining antler growth score was represented by the equation:

$$ASDS = 1 - [(SCORE - DECLINESCORE)/60]^2,$$

where SCORE is the phenotype score, and DECLINESCORE is an age-dependent value that reflects the score at which survival begins to drop. Values for DECLINESCORE of calves and yearlings were 40, and for bulls aged 2-7 were 20, 16, 12, 8, 6 and 4, respectively. For bulls $\geq$8 yrs the value of DECLINESCORE was 2. We assumed that mortality would increase as a function of antler size because the energy required to produce and carry large antlers, as well as that required to achieve and maintain dominance during rut would place large-antlered animals in a greater energy deficit during winter compared with smaller-antlered animals. Such an outcome is common among rutting males in cervids (Bowyer 1981, 1991). With these assumptions, the initial ratios of all bulls and large (>127 cm antler spread) bulls:100 cows were 80 and 34, respectively.

Based upon data from the moose population on the Kenai Peninsula (Alaska Dept. of Fish and Game, unpubl. data) we assigned a harvest rate equal to 50% of all legal bulls. We did not assume a relationship between the age of the bull and a learned ability to avoid hunters, unlike the model developed for elk (Cervus elaphus) by Thelen (1991).

Reproductive rates (calves/cow/yr) at *K* were 0.0 for calves, 0.22 for yearlings, 1.27 for ages 2-15, 0.14 for ages 16-19, and 0.0 for age 20 (Schwartz and Hundertmark 1993). To produce these rates in the model for the population at *K* we assumed that 12%

of yearlings would produce single calves, 5% would produce twins, and 83% would produce no offspring. Respective values for other age classes were 63%, 32%, and 5% for ages 2-15 yrs, and 8%, 3%, and 89% for ages 16-19 yrs To simulate changes in productivity associated with changes in population density relative to *K* we increased the twinning rate as density decreased (Franzmann and Schwartz 1985). For populations not at *K*, the twinning rate was determined by multiplying the twinning rate at *K* by the ratio of population size at *K*:current population size. The sex ratio of offspring at birth was 1:1 (Schwartz and Hundertmark 1993).

To simulate a moose population held below *K* by predation, we increased mortality rates for all sex-age groups as population size increased. Likewise we simulated additional mortality with little increase at a population size of 4,000 and with an exponential increase until it accounted for an additional 5.6% at or above *K* (10,000 animals).

Because our model was stochastic, we conducted 10 simulations of each scenario, from which we generated means and standard deviations of estimates of population and genetic composition. The original modeling exercise (Hundertmark et al. 1993) tracked populations for 50 yrs, but we extended the model to 100 yrs in this effort. Estimates of population composition and allele frequencies (Q_A) were generated from the initial population (year 0) and at 5-yr. intervals to year 100. The simulation of no harvest in a population at *K* (Model A) conducted by Hundertmark et al. (1993) was compared with simulations of selective harvest in a population at *K* (Model B), and selective harvest in a population held below *K* by predation (Model C). Comparisons between new simulations

and Model A were necessarily limited to the first 50 yrs because of the length of simulations conducted by Hundertmark et al. (1993), and are reported here to facilitate comparison with that earlier effort. Comparisons between the current two models included data from the entire 100-yr simulations.

Differences in final estimates of parameters between any two simulations were tested with a *t*-test; all comparisons were tested simultaneously and Bonferonni adjusted probabilities were reported (Wilkinson et al. 1996). Differences in parameters among all simulations were tested with ANOVA. Post-hoc tests among means within ANOVA were conducted with Bonferonni comparisons (Wilkinson et al. 1996).

The effects on model results of changes in heritability and different harvest criteria were discussed by Hundertmark et al. (1993) and Thelen (1991). Thelen (1991) also documented the response of the original model to changes in assumptions concerning genetic control of antler growth and in parameters controlling population dynamics. We believe that the model is robust with respect to perturbations in these basic functional relationships.

RESULTS

Population size after 50 yrs differed among the populations ($F_{2,27} = 1350$, $P < 0.0001$). Both harvested populations had significantly fewer animals than the unhunted population (Model A) and Model C (with predation) had significantly fewer animals than Model B (hunting at *K*). Both populations subjected to hunting (B and C) declined initially as hunting was instituted (Fig. A.2a). The population under Model B recovered from this decline as density-dependent processes brought the total size back toward *K*

where the population stabilized. The population under Model C stabilized at approximately 7,000 animals, representing an equilibrium between the greater rates of mortality and increased productivity associated with better nutrition.

Percent declines in Q_A (frequency of favorable alleles) over the first 50 yrs for Models A, B, and C were 1.1, 5.4, and 15.5, respectively (Table A.2). Estimates of Q_A in year 50 differed significantly ($F_{2,27} = 503.4$, $P < 0.0001$) among the three models, with selective harvest in Model C causing the greatest decline. In the two models involving selective harvest (B and C), allele frequencies continued to decline steadily but at different rates through the 100 yrs of the simulation (Fig. A.2b).

Total harvest under both models decreased for the first 10 yrs, primarily because the population was unhunted prior to year 1. After year 10, harvest increased initially prior to becoming stable for Model B, whereas it decreased slowly for Model C (Fig. A.2c). By year 50, the mean harvest of small bulls under Model C was significantly less than under Model B ($t = 8.35$, $d.f. = 18$, $P < 0.0001$; Table A.2). Mean harvest of large bulls also was less under Model C ($t = 10.68$, $d.f. = 18$, $P < 0.0001$; Table A.2). Moreover, the proportion of spike-fork yearlings in the harvest differed between the two scenarios (B and C). Under both models, the proportion of spike-fork yearlings increased concomitant with the decrease in harvest in the first decade; this reflected the harvest of abundant large bulls in the previously unhunted populations. Subsequently, the small bull:large bull ratio in the population prior to harvest increased slightly under Model B, but increased at a faster rate under Model C (Fig. A.2*d*). By year 100, spike-fork yearlings represented 44% of the harvest under Model B, whereas they represented 52%

of the harvest under Model C, compared with an approximate 30% share of the harvest initially.

Ratios of bulls:100 cows of hunted populations after 50 yrs were reduced significantly ($F_{2,27} = 2800$, $P < 0.0001$; Table A.2) from that of the unhunted population and differed significantly from each other although this latter difference may have no practical biological significance for management. Moreover, these ratios were well above the objective level of approximately 30 bulls:100 cows (Schwartz et al. 1992). Ratios of large bulls:100 cows also differed among the simulations ($F_{2,27} = 4592$, $P < 0.0001$), with hunting causing a marked decrease. At 50 yrs, the low-density population had a significantly higher ratio of large bulls:100 cows than did the hunted population at K (Table A.2).

The most notable difference in composition between the two hunted populations was the decrease in the number of large bulls in Model C (Fig. A.2e). After the initial decrease in the first decade, numbers of large bulls increased slightly under Model B and stabilized. Under Model C, numbers of large bulls continued to decrease. After 100 yrs, large bull:100 cow ratios for Model B (4.2 [SD 0.32]) and Model C (4.5 [SD 0.41]) did not differ ($t = -1.75$, $d.f. = 18$, $P = 0.097$), but the ratio of the population in Model C was expected to continue to decline. The number of spike-fork yearlings in each population increased at relatively constant rates (Fig. A.2f). Thus, the proportion of spike-fork yearlings in the harvest under Model C initially was less than that under Model B, but increased at a faster rate and was greater than that in Model B by year 50 (Fig. A.2d).

DISCUSSION

We caused an increase in expression of antler size via increased nutrition in a population in which density was decreased relative to *K*. This increase in phenotype initially caused an increase in the proportion of large bulls in the population (relative to a population at *K*) and a decrease in the proportion of spike-fork yearlings. These changes were short-lived, however, as changes in harvest of these groups caused changes in allele frequencies. Specifically, the decrease in number of spike-fork yearlings available for harvest caused a decrease in selection against unfavorable antler alleles. Under the original simulations conducted by Hundertmark et al. (1993), the harvest of animals with inferior genotypes (i.e., spike-fork yearlings) balanced the harvest of animals with superior genotypes (i.e., large bulls) and acted to stabilize allele frequencies over time. In the current simulation, more inferior animals grew palmated antlers as yearlings because of better available nutrition and thus were protected from harvest. This caused an increase in the frequency of unfavorable antler alleles and a corresponding decrease in the frequency of favorable ones. Moreover, animals with superior genotypes would, due to increased nutrition, spend less time in the protected class of animals and would thus obtain less of an opportunity to mate. The outcome of these changes in phenotype was an increase in selection pressure against favorable alleles and a consistent decrease in the proportion of bulls in the population with antler spreads $\geq$127 cm.

McCullough (1984) demonstrated that trophy harvest from a white-tailed deer herd was higher when both males and females were harvested. He argued that reducing population density below *K* caused an increase in nutritious forage and a corresponding

increase in age-specific antler size. The absolute decrease in number and harvest of large bulls in Model C (with predation) seemingly runs counter to this idea, but McCullough (1984) was considering harvest as a random process whereas we harvested males based on antler size. Harvest based on antler size always decreased the frequency of favorable antler alleles compared with random harvest in previous simulations (Hundertmark et al. 1993).

Changes in antler size were gradual in this simulation and likely would escape detection for a number of yrs Ultimately, however, there would be adequate time to detect and rectify the observed problems. Managers should monitor ratios of large:small bulls in their post-hunt surveys and in their harvest reports. Any significant and lasting change in this ratio not accounted for by changes in population density would likely indicate potential changes in genetic composition in the population.

We assumed that K did not change throughout the simulations; this assumption is unlikely to hold in many populations. Nonetheless, our results are informative because they demonstrate that alleles controlling antler growth are subject to a continuum of selective force ranging from balancing selection at or near K to selection against favorable alleles below K. This information is particularly relevant to moose populations in Alaska that are being considered for intensive management (Hundertmark and Schwartz 1996).

This exercise illustrates the contribution of environment to antler growth and the possible effect, given our assumptions, that this may have on management of moose populations by selective harvest. The relative position of the population with respect to K

will determine the success of selective-harvest management. The trends observed in this simulation were dramatic only when extended many years into the future, allowing time to detect and rectify problems. The true relationships between nutrition and expression of antler size in moose needs to be documented before real effects of selective harvest can be more thoroughly assessed.

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Table A.1. Percentage of bulls, by antler spread, in four age classes from the northern Kenai Peninsula, Alaska used as the starting population in this modeling exercise.

	Antler spread			
Age (yrs)	Spike/fork	<91 cm	≥91 and <127 cm	≤127 cm
1.5	60	25	15	0
2.5-3.5	0	25	60	15
4.5-5.5	0	0	60	40
≥6.5	0	0	5	95

Table A.2. Population parameters of simulated moose populations at year 50. Data for Model A taken from Hundertmark et al. (1993). Values represent means (SD) of 10 simulations. Harvest data are means of years 30-50 of the simulations.

Model	Frequency of favorable alleles	Population size	Harvest of small (spike/fork) bulls	Harvest of large (>127 cm spread) bulls	Number of bulls per 100 cows	Number of large bulls per 100 cows
A	0.490^{A}	$9{,}956^{A}$	0	0	79.4^{A}	33.5^{A}
	(0.0033)	(167)			(1.65)	(1.2)
B	0.470^{B}	$9{,}457^{B}$	140^{a}	210^{a}	43.4^{B}	4.4^{B}
	(0.0024)	(166)	(3)	(3)	(0.88)	(0.3)
C	0.420^{C}	$6{,}805^{C}$	126^{b}	195^{b}	37.2^{C}	5.1^{C}
	(0.0079)	(90)	(4)	(3)	(1.09)	(0.5)

^{A,B,C} Means within a column differ significantly (ANOVA and Bonferonni post hoc comparisons)

^{a,b} Means within a column differ significantly (*t*-test)

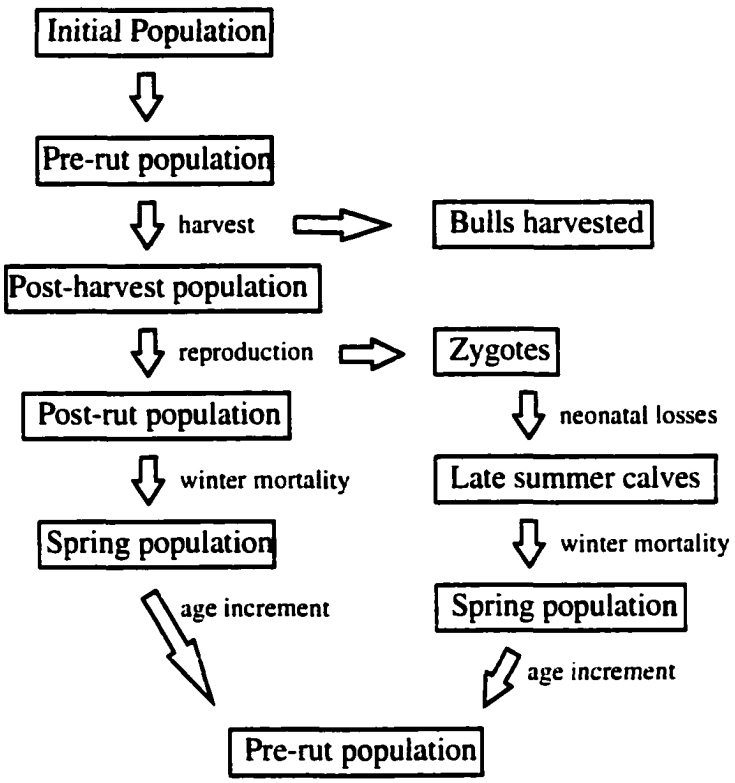

Fig. A.1. Flow chart of the stochastic model representing demographic processes occurring during a single year in a simulated population of moose (*Alces alces*).

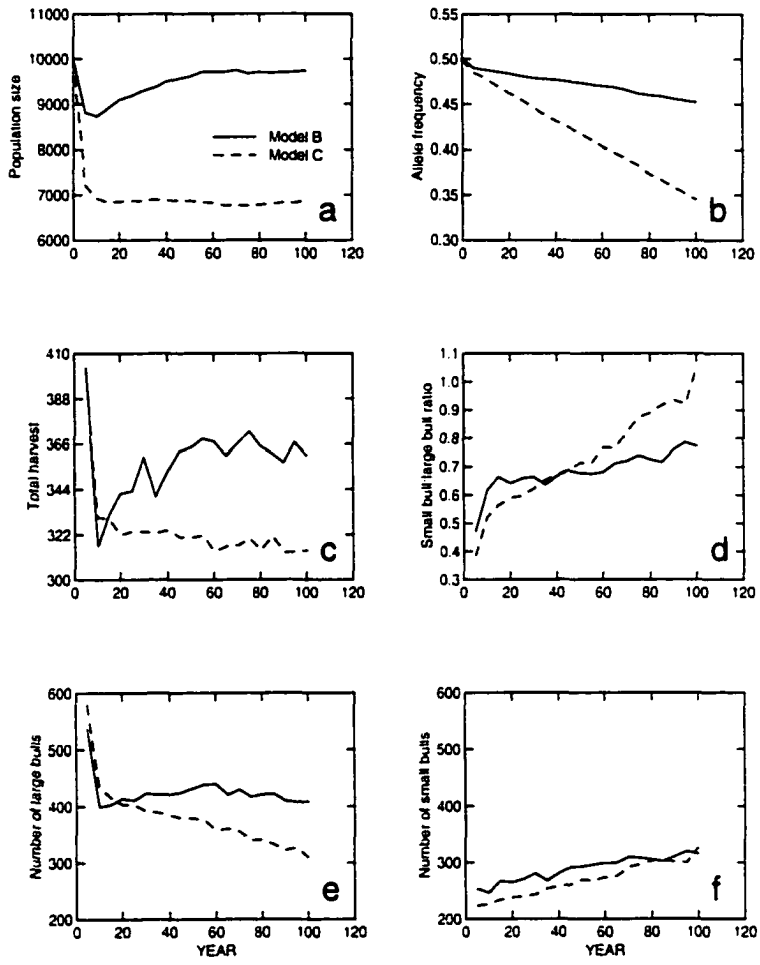

Fig. A.2. Temporal changes in a) total population size, b) frequency of favorable antler alleles, c) total harvest, d) small bull:large bull ratio prior to hunting, e) number of large bulls in the population prior to hunting, and f) number of small bulls in the population prior to hunting for simulated moose populations subjected to either harvest Model B (harvest at K) or C (harvest below K). Data represent means of 10 simulations.